

year **2009**

volume **32**

part **2**

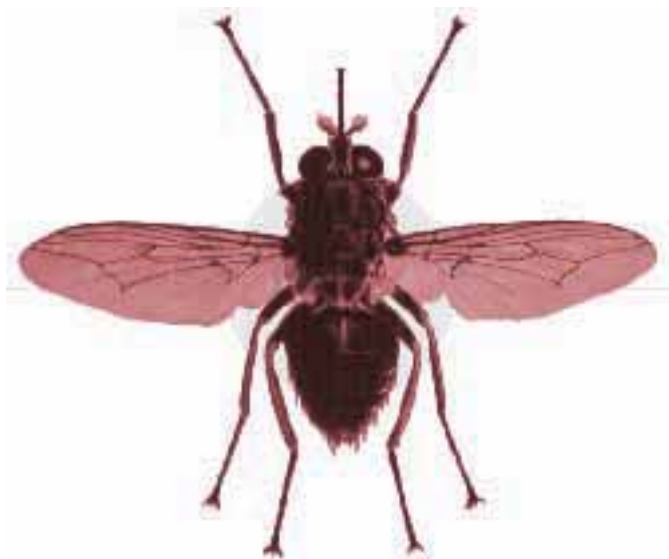
PAAT

Programme
Against
African
Trypanosomosis



ISSN 1812-2442

TSETSE AND TRYPANOSOMOSIS INFORMATION



DFID

Department for
International
Development



year **2009**

volume **32**

part **2**

PAAT

Programme

Against

African

Trypanosomosis

TSETSE AND TRYPANOSOMOSIS INFORMATION

Numbers 14961–15195

Edited by
James Dargie
Bisamberg
Austria

The designations employed and the presentation of material in this information product do not imply the expression of any opinion whatsoever on the part of the Food and Agriculture Organization of the United Nations (FAO) concerning the legal or development status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or products of manufacturers, whether or not these have been patented, does not imply that these have been endorsed or recommended by FAO in preference to others of a similar nature that are not mentioned.

ISBN 978-92-5-106641-6

All rights reserved. FAO encourages reproduction and dissemination of material in this information product. Non-commercial uses will be authorized free of charge upon request. Reproduction for resale or other commercial purposes, including educational purposes, may incur fees. Applications for permission to reproduce or disseminate FAO copyright materials and all other queries on rights and licences, should be addressed by e-mail to copyright@fao.org or to the Chief, Publishing Policy and Support Branch, Office of Knowledge Exchange, Research and Extension, FAO, Viale delle Terme di Caracalla, 00153 Rome, Italy.

© FAO 2010

TSETSE AND TRYPANOSOMOSIS INFORMATION

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

Organizations

AfDB	African Development Bank
ANDE	Agence Nationale de Développement de l'Élevage
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

Tsetse and Trypanosomosis Information

CGIAR	Consultative Group on International Agricultural Research
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIRAD-EMVT	Département d'Élevage et de Médecine Vétérinaire des Pays Tropicaux du CIRAD
CIRDES	Centre International de Recherche-Développement sur l'Élevage en Zone Subhumide
CNERV	Centre National d'Élevage 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'Élevage, 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'Élevage et de Recherches Vétérinaires
LRE	Laboratoire Régional de L'Élevage
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

Tsetse and Trypanosomosis Information

OCEAC	Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale
OGAPROV	Office Gabonais pour l'Amélioration de la Production de la Viande
OIE	Office International des Epizooties
OMVG	Organisation pour la Mise en Valeur du Fleuve Gambie
PAAT	Programme against African Trypanosomosis
PATTEC	Pan-African Tsetse and Trypanosomosis 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

	<i>Page</i>
SECTION A – NEWS	
Programme Against African Trypanosomosis: Report of the 14 th PAG Coordinators Meeting, Kampala, Uganda	1
External Evaluation of PAAT	13
SECTION B – ABSTRACTS	
1. General (including land use)	15
2. Tsetse biology	
(a) Rearing of tsetse flies	21
(b) Taxonomy, anatomy, physiology, biochemistry	21
(c) Distribution, ecology, behaviour, population studies	24
3. Tsetse control (including environmental side effects)	26
4. Epidemiology: vector-host and vector-parasite interactions	28
5. Human trypanosomosis	
(a) Surveillance	38
(b) Pathology and immunology	40
(c) Treatment	44
6. Animal trypanosomosis	
(a) Survey and distribution	50
(b) Pathology and immunology	56
(c) Trypanotolerance	59
(d) Treatment	61
7. Experimental trypanosomosis	
(a) Diagnostics	63
(b) Pathology and immunology	63
(c) Chemotherapeutics	75
8. Trypanosome research	
(a) Cultivation of trypanosomes	88
(b) Taxonomy, characterization of isolates	88
(c) Life cycle, morphology, biochemical and molecular studies	90

SECTION A – NEWS

PROGRAMME AGAINST AFRICAN TRYPANOSOMOSIS (PAAT): REPORT OF THE 14TH PAAT ADVISORY GROUP (PAG) CO-ORDINATORS MEETING, KAMPALA, UGANDA, 14-15 OCTOBER 2008

The meeting was hosted by the Ministry of Agriculture, Animal Industry and Fisheries. Mr A. A. Ilembade, Chairman of PAAT, chaired the meeting which was attended by 22 participants from international organizations (FAO, AU-IBAR, IAEA, IFAD, WHO), African-based (ICIPE) and European-based (ITM, University of Glasgow, UK) research institutions and representatives of five African countries (Ethiopia, Ghana, Kenya, Mali, Uganda), including National PATTEC Coordinators. Representatives of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC) Secretariat and Executive Committee, and of private sector entities involved in animal health were also in attendance.

The meeting was officially opened by H.E. the Minister of State for Agriculture, Animal Industry and Fisheries.

African Trypanosomosis (both the human and animal form) was highlighted as major obstacle to the socio-economic and sustainable rural and agricultural development of sub-Saharan Africa. Uganda faces almost a unique situation where the two forms of sleeping sickness (the acute form due to *Trypanosoma brucei rhodesiense* and the chronic form due to *T. b. gambiense*) coexist in the same geographic area with cattle acting as a reservoir of sleeping sickness. The Minister stressed the necessity to undertake collaborative actions in the fight against trypanosomosis and wished the PAG meeting to provide substantial contributions and guidance in this direction.

In his address, Mr A. A. Ilembade recalled the mission of PAAT and its major objectives: addressing the reduction of rural poverty, improving livelihoods of rural people and ensuring food security. The PAG meetings are occasions where policies, strategies and actions for interventions against tsetse and trypanosomosis (T&T) are discussed, harmonized and agreed upon. Only with such a common and harmonized understanding will it be possible to reach sound results for the progressive elimination of the problem posed T&T to the development of sub-Saharan Africa. PAG is also a forum for setting priorities and measures to support Africa Union Member States, IBAR and PATTEC in their efforts to promote livestock-agriculture development in tsetse intervention areas. Generally speaking, PAAT must be seen as the international platform for sub-Saharan African countries to address the problems posed by and/or related to the presence of T&T and for promoting the necessary international and multi-disciplinary dialogue among highly experienced experts for guiding policy makers, advisors, planners and researchers on all aspects of dealing with T&T. In this regard, PAAT is unique in its function of developing a UN Agency – African based partnership for the development of tsetse affected areas.

1. Review and adoption of the last PAG meeting report – A.A. Ilembade

The PAAT Chairman and participants reviewed the report of the last PAG meeting, held in Luanda, Angola, September 2007. The report was adopted unanimously. Consequently, the conclusions and recommendations of the last meeting were discussed and endorsed.

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

The participants were informed about FAO and PAAT activities since the last PAG meeting. Normative, technical and logistic assistance was provided to PAAT partner countries.

FAO plans to publish a book entitled “Guidelines for the Collection of Entomological Baseline Data for Tsetse Area-wide Integrated Pest Management” in late 2008 under the FAO Animal Health Series. The book includes the following sections:

- Section 1: Basic biology and anatomy of tsetse fly;
- Section 2: Planning and preparation of baseline data survey;
- Section 3: Implementation of baseline data survey.

Additional studies were published by FAO, such as “Standardizing Land Cover Mapping for Tsetse and Trypanosomiasis Decision Making” and “Geo-spatial Datasets and Analyses for an Environmental Approach to African Trypanosomiasis”. In addition, a collaboration with WHO has been initiated aiming at producing a global atlas of sleeping sickness foci and risk areas. All these studies provide standardized tools and data for planning the various phases of a T&T intervention campaign. In particular, the information included in the second of these publications deals with the southward shift of tsetse distribution in West Africa, and the effects of climate and demographic changes on AAT and HAT. These publications are complemented by the regular update of the PAAT Information System and the production of two issues per year of the Tsetse and Trypanosomosis Information bulletin which constitute a source and repository of historical data that are publicly available and can be downloaded. For instance, historical data sets have been used by Burkina Faso for planning T&T intervention in the context of the AfDB-PATTEC supported project.

As part of its contribution to the Millennium Development Goals (MDGs), PAAT has published a brochure entitled “On Target Against Poverty – The Programme Against African Trypanosomosis 1997 -2007”. The brochure highlights the role and contribution of PAAT to support the eight Millennium Development Goals.

FAO/PAAT have continued to provide support and assistance to capacity building activities and human resource development. In this context, training components have been included in two projects funded by IFAD i.e. “Pro-poor Packages to Enhance Policy and Decision Making against the Animal African Disease Burden in sub-Saharan Africa” (GCP/RAF/442/IFA) and “Development of Innovative Site-specific Integrated Animal Health Packages for the Rural Poor” (GCP/RAF/444/IFA) executed by FAO (the last project in partnership with ICIPE and CIRDES). Within the project funded by the Japanese Government, through UNTFHS (GCP/ETH/072/UNJ), which is being executed in the Southern Rift Valley of Ethiopia, training material for field level personnel and farmers has been produced, and livestock agents and farmers trained. Also, FAO hosted a “Tsetse and Trypanosomiasis Information System Harmonisation Session” in July 2009. Technical assistance was also provided to the 5th international course on African trypanosomoses hosted by ICIPE, Nairobi, Kenya, October 2009, and organized by the Association against Trypanosomiasis in Africa, with the support of WHO.

On methodological approaches and techniques to be used for tsetse suppression and eventually disease elimination, it was agreed to explore the feasibility of using the sequential aerosol technique (SAT) in certain areas where the AfDB-PATTEC supported projects are on

going, e.g. the Southern Rift Valley of Ethiopia. Burkina Faso and Ghana have undertaken similar initiatives.

FAO/PAAT and PAAT mandated organizations have offered their assistance to countries in developing and refining current national and regional working plans for T&T field interventions, particularly within the framework of the PATTEC initiative and the AfDB funded projects in Burkina Faso, Ethiopia, Ghana, Kenya, Mali and Uganda.

A presentation was made focusing on the mission, strategy, organizational structure and role of the various institutional components of PAAT with a view to stimulating an analysis of the Programme for possible adjustments, including on how to best operate PAAT in the future and formulate advice on strengthening the Programme.

Meeting participants were informed on the official signature of the Memorandum of Understanding between FAO and IFAH on cooperation in the establishment of standards and protocols for quality control of trypanocidal drugs. IAEA, UNIDO and UNODC also participate in this initiative.

3. Report from WHO – P. Simarro

WHO reported on human African trypanosomosis (HAT) surveillance and control programmes and on current trends in HAT epidemiology.

The cornerstones of WHO activities against sleeping sickness are (i) access to diagnosis and treatment (ii) capacity building, and (iii) guidance. In the last year, 25 endemic countries have received support only from WHO, five have received support from WHO, NGOs and through bilateral cooperation, and six countries have neither received support from WHO, NGOs, nor through bilateral cooperation. As to WHO-supported active case finding, HAT screening was carried out in the Upper West Region of Ghana (11 516 people were tested in 48 villages), in Mali, 5 408 people were screened in 11 villages and in Burkina Faso, 2 459 people in six villages were screened; support included advocacy and awareness-raising. A tsetse survey was carried out in Swaziland (see also Part 14 of this report). The latest continental and country-level figures of the number of reported cases and number of people screened were presented. In 2007, 10 446 new cases were reported to WHO from affected countries, and 2 539 372 people were screened. The Atlas of HAT was also introduced (see Part 6 below).

An update was given on the epidemiology of *T. b. gambiense* and *rhodesiense* sleeping sickness. *T. b. gambiense* accounts for 97 percent of HAT cases reported from endemic countries (over 200 foci). Humans are the main reservoir and the role of domestic and wild animals remains unclear. Disease control hinges on case detection and treatment. Targeted vector control could contribute to faster disease control. Common features of all countries where Gambian trypanosomosis is endemic are the low priority given to HAT by the weak national health systems, and the remoteness of endemic areas. In the 18 countries where the number of reported cases is very low, there is also a lack of equipment and skilled professionals. By contrast, in the six countries where the number of cases is higher, there exist National Sleeping Sickness Control Programmes, skilled health workers and equipment. For the first group of countries, an intercountry catalytic team is recommended, whereby one or two technical assistants train and assist local teams to assess former and current transmission areas, set up a passive surveillance system and monitor disease evolution. For the second group of countries, WHO's strategy is to support national HAT control teams in (i) carrying out active case-finding surveys, (ii) integrating HAT control into reinforced

health systems and (iii) monitoring, analysing and reacting to the evolving epidemiological context.

T. b. rhodesiense accounts for 3 percent of HAT cases, which are reported from over 50 foci. Livestock and wildlife are important reservoirs of infection. Disease control is based on a multisectoral integrated approach, which focuses on vector control and should involve the animal reservoir. Two epidemiological settings can be distinguished: one where cattle are the main reservoir, tsetse flies are widely distributed and the population at risk is represented by villagers and herdsman; the second where wildlife is the main reservoir, the vector is confined and people interacting with parks are the main at-risk group. In the first setting, control strategies include cattle mass treatment with curative chemotherapy (twice a year) and preventive monthly restricted application of insecticide on cattle. In the second setting, interventions against the disease require vector control, awareness-raising and provision of equipment.

The WHO representative concluded by stating that elimination of the disease is possible considering the results obtained in the course of the last few years, the epidemiological knowledge acquired, and the political will demonstrated by the African Union summit in Lomé in 2000 and the on-going Pan African Tsetse and Trypanosomiasis Eradication Campaign. However, elimination should not be confused with eradication and it requires continuous and adapted interventions. Without a proper understanding of this reality elimination may become the enemy of success.

4. Report from AU/IBAR – F. Oloo

The AU-IBAR representative outlined the report of the 33rd Executive Committee meeting of the International Scientific Council for Trypanosomiasis Research and Control (ISCTRC), held in Kampala, Uganda, 16-17 October 2008.

Details were provided on the organization and outcomes of the 29th ISCTRC conference (Luanda, Angola, 1-5 October 2007). A total of 120 abstracts were submitted to the scientific committee and 105 selected and put in the programme for presentation. Most of the participants with oral presentations attended the conference and presented papers (81 percent), as compared with 45 percent of those with posters. A total of 208 participants attended the conference. They came from 27 out of 37 affected African countries and others were mainly from Europe. Fifteen international institutions that are involved in the mandate of the conference were represented.

The ISCTRC Secretary attended the meeting of Human African Trypanosomiasis (HAT) Advocacy Advisory Group (TAG), 12-14 May 2008, upon invitation by the PATTEC Coordinator. The meeting discussed HAT advocacy and proposed a medium term (three-year) Strategic Plan to be funded by the Foundation for Innovative New Diagnostics (FIND). The mission was jointly supported by PATTEC and FIND.

Following a recommendation of the 32nd Executive Committee held in Luanda, Angola, 30 September 2007, an AU/IBAR Mission comprising a renowned veterinarian, Professor Albert Ilemobade, Chairperson of PAAT and Francis Oloo, the ISCTRC Secretary undertook a mission (19-23 January 2008) to the International Trypanotolerance Centre (ITC) to assess the steps to strengthen the Centre. The team recognized the importance of ITC in managing the trypanotolerant genetic resources in the regions and recommended that the participating stakeholders resumed the previous capacity of the Centre and appealed to the

Government of the Gambia to consider its regional status by forming a Council of Ministers to enable the participating countries to support it.

5. Report from PATTEC – H. M. Solomon

Mr Solomon provided a brief on the PATTEC Initiative as it has developed during the last 8-10 years, including the identification of T&T as a problem, the realization of the need to act and to take decisions to act, the development of an action plan, the mobilization of support and the preparation and implementation of projects.

Current PATTEC activities include consultations with governments, development of project proposals, technical workshops, resource mobilization, interstate mediation, advocacy activities, training and capacity building, monitoring and reporting, and providing coordination and support services. The pioneering efforts carried out by six countries within the first phase of the PATTEC initiative were recalled (Burkina Faso, Ghana, Mali, Ethiopia, Kenya, Uganda), together with the facilitation role played by PATTEC in the elimination campaign in the common belt of Botswana and Namibia. Project proposals for T&T elimination activities are also planned to be initiated in many other regions.

PATTEC strategies for the “final push” were also indicated, including the need for a consensus on the technical feasibility of T&T eradication, simultaneous and uninterrupted action, increased advocacy and support to action, initiation and execution of an increased number of projects, more success stories and increased coordination and harmonization.

6. PAAT Information System – G. Cecchi

Recent activities carried out in the framework of the PAAT Information System (PAAT-IS) included (i) collaboration between WHO and FAO to develop the Atlas of HAT, (ii) the publication of “Geospatial Datasets and Analyses for an Environmental Approach to African Trypanosomiasis”¹, and (iii) the development of a decision support system for T&T interventions in East Africa based on Geographic Information Systems (GIS) (see below).

The Atlas of human African trypanosomosis (HAT) is a WHO-led initiative executed in collaboration with FAO within the Programme against African Trypanosomosis (PAAT)²⁻³. The Atlas aims at geo-referencing all cases of HAT reported in sub-Saharan Africa, as well as all active screening activities. Epidemiological data are provided by National Sleeping Sickness Control Programmes (NSSCPs), Non-governmental Organizations and Research Institutes. HAT cases are geo-referenced at the village level by means of global positioning systems (GPS), databases of named locations, paper and digital maps. The experience of field workers in affected countries also contributes to the efficiency and accuracy of the geo-referencing process. The use of GIS allows disease distribution and transmission intensity to be depicted with unprecedented spatial accuracy. NSSCPs provide input and participate in the development of the maps, which are expected to become important tools to support control activities and disease monitoring and surveillance. The final outcomes as well as the input data used for the Atlas will eventually be made available in the public domain through WHO and FAO/PAAT websites. The capacity of NSSCPs for data management will be strengthened to enable regular updates of the Atlas at the national level.

¹ <http://www.fao.org/docrep/012/i0809e/i0809e00.htm>

² <http://www.ij-healthgeographics.com/content/8/1/15>

³ <ftp://ftp.fao.org/docrep/fao/article/ak244e.pdf>

The publication “Geospatial Datasets and Analyses for an Environmental Approach to African Trypanosomiasis”, due for 2009, provides a cross section of actual and potential applications of GIS in the context of interventions against tsetse and trypanosomosis (T&T). It aims to promote the sharing of knowledge and harmonisation of methodologies among the wide range of actors concerned with the T&T problem. In the first section, a selection of geospatial datasets available in the public domain is reviewed through the lens of their possible use within T&T interventions. This review is followed by three case studies from two countries affected by trypanosomosis (Burkina Faso and Botswana). The case studies provide examples of GIS applications in operational scenarios and pay particular attention to data collection, management and analysis in the context of area-wide integrated T&T management.

7. Mapping livestock-oriented production systems in tsetse infested areas in East Africa – G. Cecchi

The Intergovernmental Authority on Development (IGAD) Livestock Policy Initiative (LPI) and PAAT are carrying out a study aimed at mapping the benefits of tsetse and trypanosomosis options in East Africa. The study area includes IGAD Member States, i.e. Djibouti, Eritrea, Ethiopia, Kenya, Somalia, Sudan and Uganda. The GIS methodology, originally developed for West Africa, requires a map of livestock production systems as input. Therefore, data and GIS layers assembled for livelihood analysis (chiefly within the framework of the “household economy approach”) are used to generate a regional map of livestock-oriented production systems⁴. Pastoral, agropastoral and mixed farming systems are defined according to the ratio of livestock- to crop-derived income and they are subsequently mapped for those areas where livelihood data are available. Gaps can be filled using statistical modelling techniques based on environmental data, thus providing complete regional coverage.

The output of this exercise will be a key component of the spatial decision-support system for the IGAD region, whose goal is to identify priority areas for trypanosomosis interventions by mapping the potential economic benefits that would result from trypanosomosis removal.

8. Sleeping sickness situation (*T. b. gambiense* and *T. b. rhodesiense*) in Uganda (2000 – 2008) – A. S. L. Kakembo

The representative of the Ugandan Ministry of Health, HAT Programme, provided an update on the HAT situation in Uganda. Both forms of HAT have been occurring in two geographically distinct foci. *T. b. gambiense* is present in the North West focus (districts of Adjumani, Arua, Moyo, Yumbe and Gulu) at the border with South Sudan and the Democratic Republic of the Congo (DRC). Total population at risk in this region is estimated at two million. *T. b. rhodesiense* is present in South East focus (districts of Bugiri, Kamuli, Kayunga, Busia, Mayuge, Iganga, Mukono, Jinja, Pallisa, Soroti and Tororo) at the border with Kenya. Total population at risk in this region is estimated at ten million. Civil strife, together with human and animal population movements triggered the expansion northwards of *T. b. rhodesiense*, which resulted in outbreaks in the districts of Soroti (1998),

⁴ <ftp://ftp.fao.org/docrep/fao/article/ak791e.pdf>

Kaberaimaido (2003), Lira / Dokolo (2004) and further north to Aloï sub-county in Lira District (2006).

The lasted figures for the reported number of HAT cases were also provided (up to 2007). For *T. b. rhodesiense* a trend towards a lower number of cases was observed in the period 2005 – 2007. For *T. b. gambiense* a similar trend is reported, which could, however, be deceptive because it is accompanied by a reduction in active screening activities, which is likely to have resulted in an increased number of undiagnosed cases.

The Ugandan Government has attempted to address the impending possible merger of *T. b. rhodesiense* and *T. b. gambiense* through a private / public partnership. Other challenges include the problems brought about by the decentralisation of HAT control to districts, which is presently being redressed, and the occurrence of HAT cases in previously silent areas (i.e. Kalangala and Pallisa districts, and on the shores of Lake Albert). Surveillance systems are collapsing due to poor logistical support, competing programmes, poor prioritisation by districts and poor advocacy. The creation of new districts out of the old endemic for HAT districts (e.g. Koboko) is further straining the logistical support. This is affecting detection of cases, follow-up of patients, and timely HAT data management and sharing.

9. Ghana and the AfDB-funded T&T project: progress and achievements, and its feasibility/bottlenecks in relation to the project cycle/time frame and budget - C. Mahama

The presenter illustrated progress with baseline data collection in the AfDB-funded project in Ghana. Entomological and parasitological surveys were completed, thus allowing trypanosomosis risk to be assessed and mapped. Remote sensing data and GIS are being used to carry out an integrated environmental survey, which includes land use and land cover mapping. A GIS-based information system is being put in place by assembling all baseline data relevant for project implementation and evaluation. As concerns capacity building, 220 people have been trained, including veterinary technical officers, agricultural extension agents, community animal health workers and extension volunteers.

A HAT survey was conducted through collaboration between the Ghana Health Service, the Veterinary Services and WHO. Activities were restricted to the project area and involved the screening of 12 000 people in 48 communities.

Intervention strategies now focus on ground spraying, bait technology and the sequential aerosol technique (SAT). SAT is planned to be executed from 1 March until 15 April 2008 using deltamethrin over an area of 8 000 km² composed of narrow blocks along rivers. Environmental monitoring will be conducted alongside the suppression activities.

A distorted budget, now redressed, was cited as one of the constraints to project implementation. The strong government commitment and the excellent partnership with the AfDB were mentioned as the main strengths of the project.

10. Mali and the AfDB-funded T&T project: progress and achievements, and its feasibility/bottlenecks in relation to the project cycle/time frame and budget - A. Djiteye

In Mali, 2.5 and 2.7 million people and cattle, respectively, are exposed to the risk of trypanosomosis. 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 trypanosomosis intervention and the relatively new AfDB-funded project is a continuation of previous tsetse control campaigns. The project has an area of 37 000 km²: 15 000 km² in the Niger river basin, the peri-urban area of Bamako and 22 000 km² in the Bani river basin, from the northern limit of the tsetse distribution to the border with Burkina Faso. The eradication targets 15 000 km².

Regional meetings and communal workshops were organized for raising community awareness. Farming communities were involved through the creation of village brigades for tsetse and trypanosomosis control. Efforts were also made to involve technicians of the public and private sectors. Entomological data indicate that the average apparent tsetse reduction achieved is 97 percent in the project area.

Under the AfDB-funded project the establishment of a colony of *G. p. gambiensis* is foreseen with the aim of producing males for an SIT campaign. Studies should target the possibility of using fluorescent powder mixed with sand to mark emerging flies, and to estimate the impact of these treatments on sexual behaviour of male flies and fertility of females. Also, the quality of the irradiated and marked tsetse flies will be evaluated, first at CIRDES and then after transportation in Bamako, in order to determine the impact of transportation on tsetse fly quality.

11. Ethiopia and the AfDB-funded T&T project: progress and achievements of AfDB funded project and its feasibility/bottlenecks in relation to the project cycle/time frame and budget - T. Alemu

In Ethiopia, the AfDB-funded project is complementing the ongoing STEP project. Tsetse mass rearing activities are continued in the Arba Minch colony and in Ttororo colony (*G. fuscipes fuscipes*). In the Arba Minch colony, a decline has been observed in the colony size and in the pupae production.

Field operations include tsetse suppression and monitoring. For routine suppression four targets/km² are deployed, and 20 percent of cattle treated, while for intense suppression six targets/km² are deployed and 50 percent of cattle are treated. There is also routine and intense tsetse monitoring and reporting. For the former, two traps/km² are deployed four times a year around villages and livestock areas; for the latter, two traps/km² are deployed monthly according to the principles of area-wide pest management (i.e. targeting the whole pest population). Test releases of tsetse sterile males were carried out in the area of Arba Minch following intensive tsetse suppression.

It is estimated that the AfDB-funded project time frame, which requires achievement of eradication in the entire STEP area is highly optimistic and it will need to be revised during the mid-term review by setting more realistic, achievable targets.

The project Steering Committee discussed the issue of SAT, and recommended that STEP management should approach AU-PATTEC to carry out a SAT feasibility study in Ethiopia.

12. Kenya and the AfDB-funded T&T project: progress and achievements, its feasibility/bottlenecks in relation to the project cycle/time frame and budget - P. Olet

Kenya has a surface area of approximately 587 000 km² with a landmass of 576 000 km². The tsetse infested area covers 25 percent of the landmass. The eight species present in Kenya are distributed over five tsetse belts named after the regions in which they are located i.e. Lake

Victoria, Rift Valley, Coast Region, Central Region, and Eastern Region. Discrete belts are believed to be conducive to eradication.

The Af-DB project in Kenya has completed the baseline surveys and vector and disease suppression are ongoing by integrating livestock spraying, deployment of traps and targets, netted zero grazing units and ground spraying.

In Ruma National Park, one of the project areas, a total of 1 500 traps were installed and the average number of flies trapped per day was reduced from 80 to 0.01. Disease prevalence dropped from 20 percent to between zero and 3.8 percent. No cases of human trypanosomosis are reported. Over 150 000 sick animals have been treated with trypanocides. Communities continued to spray livestock, and maintained and installed traps in farm lands around the park. Land use guidelines are being developed to scale up crop and livestock production.

In the Meru/Mwea Game Reserve, another project area, densities were reduced from 71 to 0.3 upon the installation of 334 impregnated targets. Livestock spraying by communities is ongoing. Trypanosomosis prevalence rate in cattle varied between 0.8 percent and 15.6 percent.

Project strengths are: political good will of African Heads of State and governments, support from stakeholders including AU, African Development Bank and others, committed and skilled manpower, networks and structures in place, willing communities, availability of technologies for eradication of tsetse and trypanosomosis.

The weaknesses can be found in the insufficient and untimely allocation of funds by the government and donors, inadequate manpower and obsolete, fragmented and conflicting policies, legal and institutional framework, and the lack of empowerment of the communities in endemic areas. PATTEC has a global mandate that does not adequately address prevailing circumstances, calling for a need to customise implementation in individual countries.

13. Uganda and the AfDB-funded T&T project: progress and achievements, its feasibility/bottlenecks in relation to the project cycle/time frame and budget - L. Semakula

The baseline data collection for the AfDB-funded T&T project in Uganda is expected to start in December 2008 and to end in February 2009. A training workshop was conducted from 11 - 22 August 2008 for 26 entomologists, in order to enhance capacities for undertaking GIS-based data collection. The roles of these entomologists will be to participate and supervise the in field data collection. Trapping sites for the entomological baseline data collection were determined by using land-cover datasets, topographical maps and satellite images. 20 - 25 sites per grid in over 100 grids have thus been determined.

The refurbishment of the tsetse mass rearing facility is lagging behind because of delays by the contractor. Efforts are being made to have this work completed. Furthermore, a field insectary is being established in Buvuma Islands. Terms of reference for the environmental impact assessment for the project have been cleared by the National Environment Management Authority (NEMA), and six firms have been shortlisted and submitted to AfDB for a decision.

The AfDB approved the government requests to revise the project cost tables in order to create resources for those activities that are critical for advancing project implementation. The budget for tsetse suppression was revised from US\$ 250 500 to US\$ 2 073 000 and it is now estimated to cover 6 640 km². Funding from IAEA Technical cooperation (US\$ 297

675) has been approved for 2009 - 11, and a Country Programme Framework (CPF) has been submitted to IAEA for consideration.

14. Tsetse survey in Swaziland – R. Saini

A tsetse survey was carried out to establish the true picture of trypanosomosis in Swaziland for the country to be declared a tsetse and trypanosomosis free zone.

The study concluded that Swaziland is not a T&T free zone. No human trypanosomosis cases have been reported for more than a century. The only vector species present is *G. austeni*, which is not involved in transmission of human trypanosomosis. Therefore, Swaziland cannot be labelled as a HAT endemic country. Even though, only two cases of animal trypanosomosis have been recorded in the last nearly 30 years, the presence of *G. austeni*, which is known to transmit nagana, cannot rule out Swaziland being at risk from animal trypanosomosis (especially along the Mozambique border where the situation should be monitored carefully).

The creation of tsetse free zones in the Republic of South Africa and the southern part of Mozambique cannot ignore the presence of tsetse on the eastern border of Swaziland with Mozambique. This area could be a site for reinvasion into Mozambique and hence in any control/eradication plans, Swaziland must be included.

It was recommended that a second seasonal survey be carried out to confirm the presence of tsetse in Swaziland. This should be accompanied by a parasitological survey in order to determine the trypanosomes present in the flies and cattle (especially in the Mlawula Park area). Mkhaya Game Reserve to which the survey teams were denied access needs to be surveyed. In addition, the deep valleys along the Mozambique border need to be surveyed as *G. austeni* may be present there.

15. Tsetse, trypanosomosis and related scientific research activities at ICIPE and ITM (R. Saini, S. Geerts)

Mr Saini reported on ICIPE activities in the field of animal health, focusing on the outcomes of a recent review which indicated that the basic and applied research and demonstration (R&D) activities in ICIPE Animal Health Division are of a very good standard and innovative. The main recommendations that emerged from the review are that ICIPE should (i) continue with a diverse research scope to be able to find new and truly innovative ways to control insects and other arthropods affecting animal health, (ii) upscale successful community-based T & T control strategies in Ethiopia, (iii) upscale tsetse repellent technologies, (iv) invest in updated equipment for chemical analysis and characterisation, (v) invest in rearing facilities, (vi) broaden the scope to focus on other vector-borne animal diseases transmitted by nematoceran flies. Examples of such pathogens and insects are Rift Valley fever virus, culicine mosquito vectors, bluetongue virus and sandfly vectors, (vii) undertake in-depth studies on the effect of climate change on vectors and vector-borne disease, and (viii) undertake capacity building in order to create cadres of research and vector control specialists and managers in livestock integrated pest and veterinary management.

Mr Saini also summarised the current and future R&D thrusts at ICIPE Animal Health Division, including (i) further optimisation and validation of the tsetse repellent technology to enhance its transfer, delivery and adoption, (ii) development of baits for riverine tsetse, vectors of human sleeping sickness, (iii) community based tsetse control using the adaptive

management approach using GIS technology to identify “hot-spots” of high tsetse fly densities for strategic deployment of traps in Ethiopia, (iv) reduction of human/wildlife conflicts through effective tsetse control, (v) characterisation of odour-binding proteins and receptors of tsetse, (vi) use of entomopathogens like *Metarhizium anisopliae* to enhance tsetse suppression rates and thereafter, mop up residual populations, (vii) exploitation of recent advances in genomics and bioinformatics, together with detailed knowledge of the behaviour of the flies for optimising existing baits and for development of new innovative technologies, (viii) undertaking of any backstopping research required to implement area-wide control/eradication programmes including undertaking of detailed baseline entomological and parasitological surveys for intervention programmes, and (vix) development of baits for biting flies (tabanids, stable flies etc) that mechanically transmit trypanosomosis.

Mr Stanny Geerts reported on (i) the accreditation of ITM as an FAO Reference Centre for livestock trypanosomosis: parasite management and diagnosis, (ii) the fast development of resistance to diminazene in Zambia, and (iii) the latest developments at the International Trypanotolerance Centre (ITC), Banjul.

ITM activities in 2008 included the accreditation for molecular and *in vivo* tests for the detection of resistance to isometamidium and diminazene, and PCR-RFLP for trypanosome species identification. ITM was also active in the transfer of techniques to regional laboratories in Africa (i.e. DTVD, University of Pretoria, South Africa, and CIRDES, Burkina Faso).

An ITM study reported a five-fold increase of diminazene resistant *T. congolense* isolates over a seven-year period in Zambia (between 1996 and 2003).

Regarding the status of ITC, the Gambian Government was previously in favour of it becoming a national institute, but recommendations of the Council of ITC and of AU-IBAR have been followed and the Government now agrees that ITC should be a regional institute. As a next step, a Council of Ministers should be organized in order to create a true regional centre, to agree on new statutes, and to agree on the financial contribution of Member Countries.

16. Recommendations

(a) The PAG acknowledges the continuous support by IFAD to PAAT and welcomes the new IFAD funded project “Development of Innovative Site-specific Integrated Animal Health Packages for the Rural Poor in sub-Saharan Africa”. In the framework of this project, PAG recommends:

- To strengthen networking among information system specialists in the affected countries through closer technical collaboration with PAAT and its information system.

Action: Tsetse affected countries, PAAT.

(b) The PAG meeting commends the FAO/IAEA for the production of the book entitled “Guidelines for the Collection of Entomological Baseline Data for Tsetse Area-wide Pest Management” and recommends:

- that the book is presented in the form of an easy-to-use multi-purpose manual targeted at field technician level.

Action: FAO/IAEA.

(c) The meeting emphasised the importance of baseline data collection as a prelude to T&T interventions and recommended:

- that baseline data collection is carried out properly in areas where T&T projects will be implemented;
- that countries comply with the standardised reporting format for data collection.

Action: On going AfDB-supported projects; other countries intending to seek financial support from AfDB or other development partners implementing T&T intervention projects/programmes.

(d) The meeting recognized that baseline datasets are becoming increasingly available from countries implementing the PATTEC initiative. It therefore recommended:

- that baseline datasets are made available in the public domain (e.g. PAAT website, FAO Geonetwork, etc.).

Action: Countries implementing AfDB-funded PATTEC projects.

(e) The PAG noted the concern expressed by the PAAT Secretariat that the Strategic Plan for Advocacy Phase I: 2008 - 2011 presented by PATTEC could lead to an overlap of the mandate and leading role of WHO as regards human African trypanosomosis control. The meeting therefore recommended:

- that collaboration between WHO and PATTEC is strengthened to complement their mandates and synergise efforts;
- that WHO is engaged in revising the outputs of the Strategic Plan for Advocacy Phase I: 2008-2011.

Action: PATTEC, WHO.

(f) The meeting noted with concern the loss of staff assigned to T&T in countries through death, retirement, transfer, etc. and recommended:

- that countries invest in the training and retention of a new generation of young professionals specialised in T&T.

Action: Countries, PATTEC, PAAT.

(g) The meeting commended the BBC for producing and broadcasting the human African trypanosomosis (HAT) documentary entitled “Survival” and recommended:

- that the film be downloaded from the BBC website http://bbcworldnews.survival.tv/documentaries/sleeping_sickness.php and used extensively as advocacy material for HAT intervention(s).

Action: Countries, WHO, PAAT, PATTEC.

(h) The meeting noted with concern that the mid-term review of the AfDB-supported projects will be due in the first quarter of 2009 and that sterile males will not be available in sufficient numbers to implement SIT within the lifetime of the current projects. The meeting recommended:

- that countries urgently review their work plans and options to include tsetse suppression methods such as SAT and ground spraying that can achieve significant results within relatively short periods;
- that, as a matter of urgency, a position paper is prepared that will address environmental concerns;
- that advocacy committees are established at technical and political levels to address the concerns of environmental lobbyists.

Action: countries, PATTEC.

(i) The meeting recognized the increasing use of techniques to control biting flies for zero-grazing cattle production units, combined with bed nets for human diseases such as mosquito-transmitted malaria. The meeting recommended:

- that impact assessments are conducted of the potential synergistic health benefits to humans and their livestock and animal production.

Action: Countries, PAAT, WHO, PATTEC.

EXTERNAL EVALUATION OF PAAT

In November 2009, FAO commissioned an External Evaluation of PAAT conducted by Drs. James Dargie (Team Leader, UK), Peter Van den Bossche (Belgium) and Oumar Diall (Mali). This Evaluation set out:

- to assess the performance of the inter-Agency (i.e. FAO/AU-IBAR/IAEA/WHO) “Programme Against African Trypanosomosis” (PAAT) since its creation in 1997 by the 29th FAO Conference of that year;
- to provide a considered opinion about its continuing relevance for addressing the current and likely future needs of its stakeholders and beneficiaries against the backdrop of scientific/technical, institutional and political changes within its founding Agencies and the countries and institutions with which they partner; and
- to provide recommendations - primarily to FAO as the principal “driver” of the inter-Agency alliance but also as considered appropriate to others within and outside that alliance - for adjustments to the structures and institutional arrangements that underpin PAAT, and to the planning and implementation of the support provided to it by FAO itself as well as by the other Agencies contributing to the Programme’s Secretariat.

In carrying out the Evaluation, the Team visited FAO Headquarters, Burkina Faso, Ghana, Ethiopia and Kenya and had extensive discussions with policy and technical decision-makers dealing with tsetse and trypanosomosis control, and livestock and wider agricultural development issues within these and other organizations/institutions and countries. Discussions by telephone were held with WHO and IAEA members of the PAAT Secretariat and with the PAAT Chairman. The Team was also provided with a rich variety of written materials concerning relevant developments within and outside PAAT, and examined information available on the PAAT and related web sites. Additionally, the Team Leader had the opportunity of presenting and obtaining feedback on the Team's major findings from members of the Panel of PAAT Advisory Group (PAG) Coordinators during their 15th meeting held in Mombasa, Kenya in December 2009.

These discussions coupled with the written inputs served to shape the Team's analyses and considerations and ultimately the conclusions and recommendations it reached concerning both the past performance and future opportunities for PAAT and FAO in assisting African countries and the international community to deal effectively with the direct and indirect consequences of animal and human trypanosomosis. The Team therefore wishes to thank all concerned for sharing their knowledge, experience and perspectives, without which the considerations underpinning and the conclusions and recommendations made in their report which was recently submitted to FAO Management would not have been possible.

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

Details of the conclusions reached and of the recommendations made by the Evaluation Team will appear in the next volume of TTI.

SECTION B - ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

14961. **Gutierrez, A. P., Gilioli, G. & Baumgartner, J., 2009.** Ecosocial consequences and policy implications of disease management in East African agropastoral systems. *Proceedings of the National Academy of Sciences USA*, **106** (31): 13136-13141.

Division of Ecosystem Science, University of California, Berkeley, CA 194720, USA. [carpediem@nature.berkeley.edu].

International research and development efforts in Africa have brought ecological and social change, but analyzing the consequences of this change and developing policy to manage it for sustainable development has been difficult. This has been largely due to a lack of conceptual and analytical models to access the interacting dynamics of the different components of ecosocial systems. Here, we examine the ecological and social changes resulting from an ongoing suppression of trypanosomosis disease in cattle in an agropastoral community in southwest Ethiopia to illustrate how such problems may be addressed. The analysis combines physiologically based demographic models of pasture, cattle, and pastoralists and a bioeconomic model that includes the demographic models as dynamic constraints in the economic objective function that maximizes the utility of individual consumption under different level of disease risk in cattle. Field data and model analysis show that suppression of trypanosomosis leads to increased cattle and human populations and to increased agricultural development. However, in the absence of sound management, these changes will lead to a decline in pasture quality and increase the risk from tick-borne diseases in cattle and malaria in humans that would threaten system sustainability and resilience. The analysis of these conflicting outcomes of trypanosomosis suppression is used to illustrate the need for and utility of conceptual bioeconomic models to serve as a basis for developing policy for sustainable agropastoral resource management in sub-Saharan Africa.

14962. **Hide, G. & Tait, A., 2009.** Molecular epidemiology of African sleeping sickness. *Parasitology*, **136** (12): 1491-1500.

Centre for Parasitology and Disease, Biomedical Sciences Research Institute, School of Environment and Life Sciences, University of Salford, Salford, UK. [g.hide@salford.ac.uk].

Human sleeping sickness in Africa, caused by *Trypanosoma brucei* spp. raises a number of questions. Despite the widespread distribution of the tsetse vectors and animal trypanosomosis, human disease is only found in discrete foci which periodically give rise to epidemics followed by periods of endemicity. A key to unravelling this puzzle is a detailed knowledge of the aetiological agents responsible for different patterns of disease - knowledge that is difficult to achieve using traditional microscopy. The science of molecular epidemiology has developed a range of tools which have enabled us to accurately identify taxonomic groups at all levels (species, subspecies, populations, strains and isolates). Using these tools, we can now investigate the genetic interactions within and between populations of *Trypanosoma brucei* and gain an understanding of the distinction between human- and

non-human infective subspecies. In this review, we discuss the development of these tools, their advantages and disadvantages and describe how they have been used to understand parasite genetic diversity, the origin of epidemics, the role of reservoir hosts and the population structure. Using the specific case of *T. b. rhodesiense* in Uganda, we illustrate how molecular epidemiology has enabled us to construct a more detailed understanding of the origins, generation and dynamics of sleeping sickness epidemics.

14963. **Hotez, P. J. & Kamath, A., 2009.** Neglected tropical diseases in sub-Saharan Africa: review of their prevalence, distribution, and disease burden. *PLoS Neglected Tropical Diseases*, **3** (8): e412.

Department of Microbiology, Immunology, and Tropical Medicine, The George Washington University, Washington, D.C., USA.

The neglected tropical diseases (NTDs) are the most common conditions affecting the poorest 500 million people living in sub-Saharan Africa (SSA), and together produce a burden of disease that may be equivalent to up to one-half of SSA's malaria disease burden and more than double that caused by tuberculosis. Approximately 85 percent of the NTD disease burden results from helminth infections. Hookworm infection occurs in almost half of SSA's poorest people, including 40-50 million school-aged children and 7 million pregnant women in whom it is a leading cause of anaemia. Schistosomiasis is the second most prevalent NTD after hookworm (192 million cases), accounting for 93 percent of the world's number of cases and possibly associated with increased horizontal transmission of HIV/AIDS. Lymphatic filariasis (46-51 million cases) and onchocerciasis (37 million cases) are also widespread in SSA, each disease representing a significant cause of disability and reduction in the region's agricultural productivity. There is a dearth of information on Africa's non-helminth NTDs. Human African trypanosomosis and visceral leishmaniasis, affect almost 100 000 people, primarily in areas of conflict in SSA where they cause high mortality, and where trachoma is the most prevalent bacterial NTD (30 million cases). However, there are little or no data on some very important protozoan infections, e.g., amebiasis and toxoplasmosis; bacterial infections, e.g., typhoid fever and non-typhoidal salmonellosis, the tick-borne bacterial zoonoses, and non-tuberculosis mycobacterial infections; and arboviral infections. Thus, the overall burden of Africa's NTDs may be severely underestimated. A full assessment is an important step for establishing disease control priorities, particularly in Nigeria and the Democratic Republic of Congo, where the greatest number of NTDs may occur.

14964. **Ilemobade, A. A., 2009.** Tsetse and trypanosomosis in Africa: the challenges, the opportunities. *Onderstepoort Journal of Veterinary Research*, **76** (1): 35-40.

Upline Resources Foundation, P.O. Box 1308, Akure, Ondo State 34001, Nigeria.

Tsetse-fly and the disease it transmits, trypanosomosis, remain an enormous disease challenge in the 37 countries of sub-Saharan Africa where the impact continues to be manifest in disease burden, increased level of poverty and decreased agricultural productivity. The impact also extends over an estimated 10 million km² (a third of the

African continent) of land area, a third of which contains some well-watered parts of the continent, thus denying humans and livestock of potentially rich arable and pastureland. The disease is a threat to an estimated 50 million people and 48 million cattle with estimated annual losses in cattle production alone of 1-1.2 billion US\$. These losses are due to stock mortality and depressed productivity, which may be of meat, milk, reproduction or traction. Beyond its direct effects on humans and livestock is its impact on African agriculture and the livelihood of the rural population in the affected countries, the fly and the disease influence where people decide to live, how they manage their livestock, and the intensity and the mix of crop agriculture. The combined effects result in changes in land use and environment which may, in turn, affect human welfare and increase the vulnerability of agricultural activity. Trypanosomosis is, therefore, both a public health and an agricultural development constraint. The challenges that the elimination or control of tsetse fly and trypanosomosis pose as well as the opportunities to develop appropriate intervention technologies are discussed in this presentation.

14965. **Karanis, P. & Ongerth, J., 2009.** LAMP--a powerful and flexible tool for monitoring microbial pathogens. *Trends in Parasitology*, **25** (11): 498-499.

Medical School, University of Cologne, Department of Anatomy, Institute II, Medical and Molecular Parasitology Laboratory, Centre of Anatomy, Institute II, Kerpener Str. 62, 50937 Cologne, Germany; Civil, Mining and Environmental Engineering, University of Wollongong, Wollongong NSW 2522 Australia. [Panagiotis.Karanis@uk-koeln.de].

Loop-mediated isothermal amplification (LAMP) is one of the nucleic acid amplification tests (NATs) available for organism identification applications in various fields such as infection diagnosis. It has been commonly described as a novel method, yet over 250 publications have appeared in less than 10 years since its original description. LAMP has been applied to produce highly specific and sensitive amplification of DNA or RNA from virtually every corner of the biological world, including prokaryotes and eukaryotes, plant and animal tissue. Among published studies, the majority are descriptions of the development and application of LAMP to detect human pathogens including viruses, bacteria, protozoa and fungi. Briefly, LAMP employs four primers recognizing six independent sequences of the selected target gene for initiation, with four sequences subsequently used for amplification and visualization of the amplified product. It uses a robust polymerase (BST) to amplify target DNA (or RNA by inclusion of reverse transcriptase) proceeding to an autocycling strand displacement mechanism, at a constant temperature, producing detectable product in approximately 1 h. Defining features of LAMP identified through its relatively short period of development include the following: it is highly selective (i.e. able to distinguish between organism subtypes) and highly sensitive, often demonstrated to amplify from a single copy or from a single organism. It is robust, with reagents stable at ambient temperature for up to two weeks, and consistently insensitive to extraneous nucleic acids or interference from sample or media components that are problematic for other NATs. The procedure is rapid and is able to amplify from a single copy to 10^9 in 1h at constant temperature, typically in the range of 60-70 °C. Requirements for sample processing and LAMP application are relatively simple, not requiring high technical skill or sophisticated equipment. Issues such as contamination and post-amplification handling, spatial separation

of work areas and direct comparison of LAMP with real-time PCR continue to be elucidated by new and innovative work. Further prospective validation in field settings will be important to broadening LAMP application and acceptance. In studies, particularly over the past five years, investigators have used different approaches to gain higher levels of resolution among closely related targets. Different primer sets were used to distinguish between four species of trypanosomes: *Trypanosoma brucei gambiense*, *T. congolense*, *T. cruzi* and *T. evansi*. A multiplex LAMP combining four primer sets in a single LAMP assay mixture was used to distinguish two species of *Babesia* (*B. bovis* and *B. bigemina*), with separation provided by subsequent *EcoRI* digestion and electrophoresis. However, restriction digestion after LAMP presents a risk for contamination that must be avoided. The increasing application of LAMP to nucleic acid extracts of unpurified samples or even to samples without nucleic acid extraction demonstrates its general insensitivity to extraneous materials other than the target. Arbovirus and *Plasmodium* (oocyst and sporozoite or other stage DNA) have been identified in whole mosquitoes processed only by tissue grinding. Hepatitis A virus, *Cryptosporidium* oocyst and *Toxoplasma* oocyst DNA have been detected efficiently in crude faecal nucleic acid extracts. The specificity and sensitivity of detection do not seem to be impaired by LAMP processing conditions or sample type, including whole blood, boiled or card-processed blood, serum, sputum and crudely processed tissue samples. However, regarding *Plasmodium* spp. detection, malaria diagnosis by LAMP requires further prospective validation to establish sensitivity. Further research towards optimizing and simplifying template production methods will also be important. LAMP is an emerging technology in the field of parasitology and further efforts in ongoing work should soon provide more comparative sensitivity results in the diagnosis of parasitic diseases. The potential for broad applicability of LAMP derives from the characteristics described above. The rapid development of many and varied applications are a product of these characteristics. Continued development will progress based on the ingenuity of individual investigators and clinicians and on opportunities to utilize LAMP characteristics to solve organism diagnostic identification problems.

14966. **Magez, S. & Radwanska, M., 2009.** African trypanosomiasis and antibodies: implications for vaccination, therapy and diagnosis. *Future Microbiology*, **4**: 1075-1087.

Department of Molecular & Cellular Interactions, Flanders Institute for Biotechnology, Rijvisschestraat 120, B-9052 Ghent, Belgium. [stemagez@vub.ac.be].

African trypanosomosis causes devastating effects on human populations and livestock herds in large parts of sub-Saharan Africa. Control of the disease is hampered by the lack of any efficient vaccination results in a field setting, and the severe side effects of current drug therapies. In addition, with the exception of *Trypanosoma brucei gambiense* infections, the diagnosis of trypanosomosis has to rely on microscopic analysis of blood samples, as other specific tools are non-existent. However, new developments in biotechnology, which include loop-mediated isothermal amplification as an adaptation to conventional PCR, as well as the antibody engineering that has allowed the development of nanobody technology, offer new perspectives in both the detection and treatment of trypanosomosis. In addition, recent data on parasite-induced B-cell memory destruction offer new insights into mechanisms of

vaccine failure, and should lead us towards new strategies to overcome trypanosome defences operating against the host immune system.

14967. **Rosenblatt, J. E., 2009.** Laboratory diagnosis of infections due to blood and tissue parasites. *Clinical Infectious Diseases*, **49** (7): 1103-1108.

Division of Clinical Microbiology, Hilton 470, Mayo Clinic, Rochester, MN 55905, USA. [rosenblatt.jon@mayo.edu].

Microscopy remains the cornerstone of the laboratory diagnosis of infections due to blood and tissue parasites. Examination of thick and thin peripheral blood smears stained with Giemsa or other appropriate stains is used for detection and identification of species of *Plasmodium*, *Babesia*, *Trypanosoma*, *Brugia*, *Mansonella*, and *Wuchereria*. Even in the hands of well-trained technologists, diagnosis may be hampered by the sparseness of organisms on the slide and by the subjective nature of differentiating similar-appearing organisms. Microscopy and/or culture of ulcer, bone marrow, tissue aspirate, and biopsy samples are useful for the diagnosis of African trypanosomosis, onchocerciasis, trichinosis, and leishmaniasis. Serologic assays are available for the diagnosis of a number of these infections, but none of these assays is sensitive or specific enough to be used on their own to establish a diagnosis. In particular, assays for the diagnosis of infection with a particular helminth will often cross-react with antibodies to a different helminth. Very sensitive polymerase chain reaction assays have been developed for a number of these parasites and are available from the Centers for Disease Control and Prevention and from several referral laboratories.

14968. **Shaw, A. P., 2009.** Assessing the economics of animal trypanosomosis in Africa—history and current perspectives. *Onderstepoort Journal of Veterinary Research*, **76** (1): 27-32.

A. P. Consultants, Upper Cottage, Abbotts Ann, Andover, SP11 7BA, UK.

Finding appropriate ways of dealing with the problem of tsetse and trypanosomosis will be an important component of efforts to alleviate poverty in Africa. This article reviews the history of economic analyses of the problem, starting with the use of cost to guide choice of technique for tsetse control in the 1950s, followed by work in the 1970s and 1980s linking these to the impact of the disease on livestock productivity, and in the 1990s to its wider impact. In the current situation, with limited resources and a range of techniques for controlling or eliminating tsetse, the cost implications of choosing one technique or another are important and a recent study reviewed these costs. A novel approach to assessing the potential benefits from removing trypanosomosis by creating “money maps” showed that high losses from animal trypanosomosis currently occur in areas with high cattle population densities on the margins of the tsetse distribution and where animal traction is an important component of farming systems. Given the importance of the decisions to be made in the next decade, when prioritizing and choosing techniques for dealing with tsetse and trypanosomosis, more work needs to be done underpinning such mapping exercises and estimating the true cost and likely impact of planned interventions.

14969. **Vale, G. A., 2009.** Prospects for controlling trypanosomosis. *Onderstepoort Journal of Veterinary Research*, **76** (1): 41-45.

Southern African Centre for Epidemiological Modelling and Analysis,
University of Stellenbosch, Stellenbosch, South Africa.

The best technical package for the future comprises trypanocidal drugs for temporary relief and the use of insecticide-treated cattle, artificial baits and aerial spraying to attack the vector, to give more lasting security. Whether this can speed the previously slow progress will depend on overcoming past hindrances to tsetse control: sporadic support, disputes over its desirability, difficulties of sustaining international operations, and poor planning in some instances. The Pan-African Tsetse and Trypanosomiasis Campaign intends to speed the progress but will fail unless it improves its image by breaking its association with the sterile insect technique and quickly executing some cheap and effective operations in large areas. Even then, there could be severe brakes due to Africa's political and financial instability. Overall, the pace of control is likely to increase, but perhaps only a little.

14970. **Welburn, S. C., Maudlin, I. & Simarro, P. P., 2009.** Controlling sleeping sickness - a review. *Parasitology*, **136** (14): 1943-1949.

Centre for Infectious Diseases, College of Medicine and Veterinary Medicine,
The University of Edinburgh, Summerhall, Edinburgh, EH9 1QH, UK.

Following a period characterized by severe epidemics of sleeping sickness, restoration of effective control and surveillance systems has raised the question of eliminating the disease from sub-Saharan Africa. Given sufficient political and financial support, elimination is now considered a reasonable aim in countries reporting zero or less than 100 cases per year. This success may lead health authorities across the affected region to downgrade the disease from "neglected" to simply being ignored. In view of the significant levels of under-reporting of sleeping sickness mortality in rural communities, this could be a short-sighted policy. Loss of capacity to deal with new epidemics, which can arise as a consequence of loss of commitment or civil upheaval, would have serious consequences. The present period should be seen as a clear opportunity for public-private partnerships to develop simpler and more cost-effective tools and strategies for sustainable sleeping sickness control and surveillance, including diagnostics, treatment and vector control.

14971. **Wilkinson, S. R. & Kelly, J. M., 2009.** Trypanocidal drugs: mechanisms, resistance and new targets. *Expert Reviews in Molecular Medicine*, **11**: e 31.

Queen Mary Pre-Clinical Drug Discovery Group, School of Biological and
Chemical Sciences, Queen Mary University of London, London E1 4NS, UK.
[s.r.wilkinson@qmul.ac.uk].

The protozoan parasites *Trypanosoma brucei* and *Trypanosoma cruzi* are the causative agents of African trypanosomosis and Chagas disease, respectively. These are debilitating infections that exert a considerable health burden on some of the poorest people on the planet. Treatment of trypanosome infections is dependent on a small number of drugs that have

limited efficacy and can cause severe side effects. Here, we review the properties of these drugs and describe new findings on their modes of action and the mechanisms by which resistance can arise. We further outline how a greater understanding of parasite biology is being exploited in the search for novel chemotherapeutic agents. This effort is being facilitated by new research networks that involve academic and biotechnology/pharmaceutical organizations, supported by public-private partnerships, and is bringing a new dynamism and purpose to the search for trypanocidal agents.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

14972. **Akoda, K., Van den Bossche, P., Marcotty, T., Kubi, C., Coosemans, M., De Deken, R. & Van den Abbeele, J., 2009.** Nutritional stress affects the tsetse fly's immune gene expression. *Medical and Veterinary Entomology*, **23** (3): 195-201.

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

Tsetse-transmitted trypanosomosis poses a serious threat to human and animal health in sub-Saharan Africa. The majority of tsetse flies (*Glossina* spp.) in a natural population will not develop a mature infection of either *Trypanosoma congolense* or *Trypanosoma brucei* sp. because of refractoriness, a phenomenon that is affected by different factors, including the tsetse fly's immune defence. Starvation of tsetse flies significantly increases their susceptibility to the establishment of a trypanosome infection. This paper reports the effects of nutritional stress (starvation) on (a) uninduced baseline levels of gene expression of the antimicrobial peptides attacin, defensin and cecropin in the tsetse fly, and (b) levels of expression induced in response to bacterial (*Escherichia coli*) or trypanosomal challenge. In newly emerged, unfed tsetse flies, starvation significantly lowers baseline levels of antimicrobial peptide gene expression, especially for attacin and cecropin. In response to trypanosome challenge, only non-starved older flies showed a significant increase in antimicrobial peptide gene expression within five days of ingestion of a trypanosome-containing bloodmeal, especially with *T. brucei* bloodstream forms. These data suggest that a decreased expression of immune genes in newly hatched flies or a lack of immune responsiveness to trypanosomes in older flies, both occurring as a result of fly starvation, may be among the factors contributing to the increased susceptibility of nutritionally stressed tsetse flies to trypanosome infection.

14973. **Caljon, G., Broos, K., De Goeysse, I., De Ridder, K., Sternberg, J. M., Coosemans, M., De Baetselier, P., Guisez, Y. & Den Abbeele, J. V., 2009.** Identification of a functional antigen5-related allergen in the saliva of a blood feeding insect, the tsetse fly. *Insect Biochemistry and Molecular Biology*, **39** (5-6): 332-341.

Department of Parasitology, Institute of Tropical Medicine, Antwerp, Belgium.

Our previous screening of a *Glossina morsitans morsitans* lamdagt11 salivary gland expression library with serum of a tsetse fly exposed rabbit identified a cDNA encoding tsetse antigen5 (TAg5, 28.9 kDa), a homologue of antigen5 sting venom allergens. Recombinant TAg5 was produced in Sf9 cells in order to assess its immunogenic properties in humans. Plasma from a patient that previously exhibited anaphylactic reactions against tsetse fly bites contained circulating anti-TAg5 and anti-saliva IgEs. In a significant proportion of plasma samples of African individuals, TAg5 and saliva binding IgEs (respectively 56 and 65 percent) can be detected. Saliva, harvested from flies that were subjected to TAg5- specific RNA interference (RNAi), displayed significantly reduced IgE binding potential. Allergenic properties of TAg5 and tsetse fly saliva were further illustrated in immunized mice, using an immediate cutaneous hypersensitivity and passive cutaneous anaphylaxis assay. Collectively, TAg5 was illustrated to be a tsetse fly salivary allergen, demonstrating that antigen5-related proteins are represented as functional allergens not only in stinging but also in blood feeding insects.

14974. **Childs, S. J., 2009.** A model of pupal water loss in *Glossina*. *Mathematical Biosciences*, **221** (2): 77-90.

South African Centre for Epidemiological Modelling and Analysis, University of Stellenbosch, Stellenbosch 7600, South Africa. [schild@sun.ac.za].

The results of a long-established investigation into pupal transpiration are used as a rudimentary data set. These data are then generalized to all temperatures and humidities by invoking the property of multiplicative separability, as well as by converting established relationships in terms of constant humidity at fixed temperature, to alternatives in terms of a calculated water loss. In this way a formulation which is a series of very simple, first order, ordinary differential equations is devised. The model is extended to include a variety of *Glossina* species using their relative surface areas, their relative pupal and puparial loss rates and their different 4th instar excretions. The resulting computational model calculates total, pupal water loss, consequent mortality and emergence. Remaining fat reserves are a more tenuous result. The model suggests that, while conventional wisdom is often correct in dismissing variability in transpiration-related pupal mortality as insignificant, the effects of transpiration can be profound under adverse conditions and for some species, in particular. The model demonstrates how two gender effects, the more significant one at the drier extremes of tsetse fly habitat, might arise. The agreement between calculated and measured critical water losses suggests very little difference in the behaviour of the different species.

14975. **Claes, F., Vodnala, S. K., van Reet, N., Boucher, N., Lunden-Miguel, H., Baltz, T., Goddeeris, B. M., Buscher, P. & Rottenberg, M. E., 2009.** Bioluminescent imaging of *Trypanosoma brucei* shows preferential testis dissemination which may hamper drug efficacy in sleeping sickness. *PLoS Neglected Tropical Diseases*, **3** (7): e486.

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

Monitoring *Trypanosoma* spread using real-time imaging *in vivo* provides a fast method to evaluate parasite distribution especially in immunoprivileged locations. Here, we

generated monomorphic and pleomorphic recombinant *Trypanosoma brucei* expressing the *Renilla* luciferase. *In vitro* luciferase activity measurements confirmed the uptake of the coelenterazine substrate by live parasites and light emission. We further validated the use of *Renilla* luciferase-tagged trypanosomes for real-time bioluminescent *in vivo* analysis. Interestingly, a preferential testis tropism was observed with both the monomorphic and pleomorphic recombinants. This is of importance when considering trypanocidal drug development, since parasites might be protected from many drugs by the blood-testis barrier. This hypothesis was supported by our final study of the efficacy of treatment with trypanocidal drugs in *T. brucei*-infected mice. We showed that parasites located in the testis, as compared to those located in the abdominal cavity, were not readily cleared by the drugs.

14976. **Terblanche, J. S., Clusella-Trullas, S., Deere, J. A., Van Vuuren, B. J. & Chown, S. L., 2009.** Directional evolution of the slope of the metabolic rate-temperature relationship is correlated with climate. *Physiological and Biochemical Zoology*, **82** (5): 495-503.

Department of Conservation Ecology and Entomology, Stellenbosch University, Private Bag X1, Matieland 7602, South Africa. [jst@sun.ac.za].

The evolution of metabolic rate-temperature (MR-T) reaction norms is of fundamental importance to physiological ecology. Metabolic cold adaptation (MCA) predicts that populations or species from cooler environments will have either a higher metabolic rate at a common temperature or steeper MR-T relationships, indicating greater sensitivity of respiratory metabolism to temperature. Support for MCA has been found in some insect species by comparing species or populations differing in latitude. However, the generality of these findings are contentious, with most studies either unable to account for phenotypic plasticity or the evolutionary relatedness of species or populations. Hence, the importance of MCA is vigorously debated from both evolutionary and ecological perspectives. Furthermore, few species, particularly from tropical environments, have been shown to differ in MR-T sensitivity along altitudinal temperature gradients. Here, using four populations of tsetse flies (*Glossina pallidipes*, Diptera: *Glossinidae*) from thermally distinct geographic regions, we test the hypothesis that there is evolved variation in MR-T relationships to cold climates. We found that a high-altitude equatorial population from a cool habitat has a steeper MR-T reaction norm. By contrast, other populations from warmer environments in East Africa do not differ with respect to their MR-T reaction norms. Squared-change parsimony analyses, based on the combined mitochondrial 16S rDNA ribosomal subunit and cytochrome c oxidase subunit I (COI), support the hypothesis of adaptive differentiation of MR-T reaction norms in the cool-climate population. Seasonal adjustments or laboratory-temperature-induced phenotypic plasticity changed the intercept of the reaction norm rather than the slope, and thus the observed intraspecific variation in slopes of MR-T reaction norms could not be accounted for by phenotypic plasticity. These results therefore suggest evolutionary adaptation of MR-T reaction norms to cool climates (<22 °C) in tsetse and provide novel support for MCA within an insect species.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

14977. **Abd-Alla, A. M., Vlak, J. M., Bergoin, M., Maruniak, J. E., Parker, A., Burand, J. P., Jehle, J. A. & Boucias, D. G., 2009.** *Hytrosaviridae*: a proposal for classification and nomenclature of a new insect virus family. *Archives of Virology*, **154** (6): 909-918.

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

Salivary gland hypertrophy viruses (SGHVs) have been identified from different dipteran species, such as the tsetse fly *Glossina pallidipes* (GpSGHV), the housefly *Musca domestica* (MdSGHV) and the narcissus bulbfly *Merodon equestris* (MeSGHV). These viruses share the following characteristics: (i) they produce non-occluded, enveloped, rod-shaped virions that measure 500-1 000 nm in length and 50-100 nm in diameter; (ii) they possess a large circular double-stranded DNA (dsDNA) genome ranging in size from 120 to 190 kbp and having G + C ratios ranging from 28 to 44 percent; (iii) they cause overt salivary gland hypertrophy (SGH) symptoms in dipteran adults and partial to complete sterility. The available information on the complete genome sequence of GpSGHV and MdSGHV indicates significant co-linearity between the two viral genomes, whereas no co-linearity was observed with baculoviruses, ascoviruses, entomopoxviruses, iridoviruses and nudiviruses, other large invertebrate DNA viruses. The DNA polymerases encoded by the SGHVs are of the type B and closely related, but they are phylogenetically distant from DNA polymerases encoded by other large dsDNA viruses. The great majority of SGHV ORFs could not be assigned by sequence comparison. Phylogenetic analysis of conserved genes clustered both SGHVs, but distantly from the nudiviruses and baculoviruses. On the basis of the available morphological, (patho) biological, genomic and phylogenetic data, we propose that the two viruses are members of a new virus family named *Hytrosaviridae*. This proposed family currently comprises two unassigned species, *G. pallidipes* salivary gland hypertrophy virus and *M. domestica* salivary gland hypertrophy virus, and a tentative unassigned species, *M. equestris* salivary gland hypertrophy virus. Here, we present the characteristics and the justification for establishing this new virus family.

14978. **Briceno, R. D. & Eberhard, W. G., 2009.** Experimental demonstration of possible cryptic female choice on male tsetse fly genitalia. *Journal of Insect Physiology*, **55** (11): 989-996.

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

A possible explanation for one of the most general trends in animal evolution - rapid divergent evolution of animal genitalia - is that male genitalia are used as courtship devices that influence cryptic female choice. But experimental demonstrations of stimulatory effects of male genitalia on female reproductive processes have generally been lacking. Previous studies of female reproductive physiology in the tsetse fly *Glossina morsitans* suggested that stimulation during copulation triggers ovulation and resistance to remating. In this study we altered the form of two male genital structures that squeeze the female's abdomen

rhythmically in *G. morsitans centralis* and induced, as predicted, cryptic female choice against the male: sperm storage decreased, while female remating increased. Further experiments in which we altered the female sensory abilities at the site contacted by these male structures during copulation, and severely altered or eliminated the stimuli the male received from this portion of his genitalia, suggested that the effects of genital alteration on sperm storage were due to changes in tactile stimuli received by the female rather than altered male behaviour. These data support the hypothesis that sexual selection by cryptic female choice has been responsible for the rapid divergent evolution of male genitalia in *Glossina*; limitations of this support are discussed. It appears that a complex combination of stimuli triggers female ovulation, sperm storage, and remating, and different stimuli affect different processes in *G. morsitans*, and that the same processes are controlled differently in *G. pallidipes*. This puzzling diversity in female triggering mechanisms may be due to the action of sexual selection.

14979. **Dyer, N. A., Furtado, A., Cano, J., Ferreira, F., Odete Afonso, M., Ndong-Mabale, N., Ndong-Asumu, P., Centeno-Lima, S., Benito, A., Weetman, D., Donnelly, M. J. & Pinto, J., 2009.** Evidence for a discrete evolutionary lineage within Equatorial Guinea suggests that the tsetse fly *Glossina palpalis palpalis* exists as a species complex. *Molecular Ecology*, **18** (15): 3268-3282.

Vector Group, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, UK.[ndyer@liv.ac.uk].

Tsetse flies of the palpalis group are major vectors of Human African Trypanosomosis in Africa. Accurate knowledge of species identity is essential for vector control. Here, we combine ribosomal internal transcribed spacer 1 (ITS1), mitochondrial cytochrome oxidase 1 (CO1) and microsatellites to determine the population structure and phylogenetic relations of *Glossina p. palpalis* in Equatorial Guinea. CO1 sequence data suggest that *G. p. palpalis* in Equatorial Guinea is a distinct subspecies from previously described *G. p. palpalis* in West Africa and Democratic Republic of Congo. *Glossina p. palpalis* in Equatorial Guinea and DRC share a common ancestor which diverged from West African *G. p. palpalis* around 1.9 Ma. Previous ITS1 length polymorphism data suggested the possible presence of hybrids in Equatorial Guinea. However, ITS1 showed incomplete lineage sorting compared with clearly defined CO1 groups, and data from 12 unlinked microsatellites provided no evidence of hybridization. Microsatellite data indicated moderate but significant differentiation between the populations analysed (Rio Campo, Mbini and Kogo). Moreover, unlike previous studies of *G. p. palpalis*, there was no evidence for heterozygote deficiency, presence of migrants or cryptic population structure. Variance effective population size at Rio Campo was estimated at 501-731 assuming eight generations per year. This study of the population genetics of *G. p. palpalis* in Central Africa provides the first estimate of genetic differentiation between geographically separated *G. p. palpalis* populations.

14980. **Geiger, A., Fardeau, M. L., Grebaut, P., Vatunga, G., Josenando, T., Herder, S., Cuny, G., Truc, P. & Ollivier, B., 2009.** First isolation of *Enterobacter*, *Enterococcus*, and *Acinetobacter* spp. as inhabitants of the tsetse fly (*Glossina palpalis palpalis*) midgut. *Infection, Genetics and Evolution*, **9** (6): 1364-1370.

UMR 177, IRD-CIRAD, CIRAD TA A-17/G, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France. [anne.geiger@mpl.ird.fr].

This paper reports the first evidence of the presence of bacteria other than the three previously described as symbionts (*Wigglesworthia glossinidia*, *Wolbachia*, and *Sodalis glossinidius*), in the midgut of *Glossina palpalis palpalis*, the tsetse fly vector of the chronic form of human African trypanosomosis in sub-Saharan African countries. Based on the morphological, nutritional, physiological, and phylogenetic results, we identified *Enterobacter*, *Enterococcus*, and *Acinetobacter* spp. as inhabitants of the midgut of the tsetse fly from Angola. *Enterobacter* spp. was the most frequently isolated. The role of these bacteria in the gut in terms of vector competence of the tsetse fly is discussed, as is the possibility of using these bacteria to produce *in situ* trypanolytic molecules.

14981. **Gomes, J., Leao, C., Ferreira, F., Afonso, M. O., Santos, C., Josenando, T., Seixas, J., Atouguia, J. & Centeno-Lima, S., 2009.** Molecular identification of *T. brucei* s.l. in tsetse flies after long-term permanence in field traps. *Journal of Infection in Developing Countries*, **3** (9): 735-738.

Unidade de Clinica das Doencas Tropicais and Centro de Malaria e outras Doencas Tropicais-LA, Universidade Nova de Lisboa, Lisbon, Portugal.

Tsetse flies (*Glossina* spp.) are responsible for the transmission of trypanosomes, agents of animal and Human African Trypanosomosis (HAT). These diseases are associated with considerable animal and human economical loss, morbidity and mortality. The correct identification of trypanosomes species infecting tsetse flies is crucial for adequate control measures. Identification presently requires technically difficult, cumbersome and expensive on-site fly dissection. To obviate this difficulty we explored the possibility of correctly identifying trypanosomes in tsetse collected, under field conditions, only for number determination. Tsetse flies that remained exposed for weeks in field traps in the Vista Alegre HAT focus in Angola, were obtained. The flies were not dissected on site and were stored at room temperature for months. DNA extraction using the whole tsetse bodies and PCR analysis were performed in 73 randomly chosen flies. Despite the extensive degradation of the tsetse, DNA extraction was conducted successfully in 62 out of the 73 flies. PCR analysis detected the presence of *T. brucei* s.l DNA in 3.2 percent of the tsetse. It is concluded that this approach could be cost-effective and suitable for vector related HAT control activities in the context of countries where entomologically trained personnel are missing and financial resources are limited.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also:32: 14969, 14990].

14982. **Lindh, J. M., Torr, S. J., Vale, G. A. & Lehane, M. J., 2009.** Improving the cost-effectiveness of artificial visual baits for controlling the tsetse fly *Glossina fuscipes fuscipes*. *PLoS Neglected Tropical Diseases*, **3** (7): e474.

Vector Group, Liverpool School of Tropical Medicine, Liverpool, UK.

Tsetse flies, which transmit sleeping sickness to humans and nagana to cattle, are commonly controlled by stationary artificial baits consisting of traps or insecticide-treated screens known as targets. In Kenya, the use of electrocuting sampling devices showed that the numbers of *Glossina fuscipes fuscipes* (Newstead) visiting a biconical trap were nearly double those visiting a black target of 100 cm x 100 cm. However, only 40 percent of the males and 21 percent of the females entered the trap, whereas 71 percent and 34 percent, respectively, alighted on the target. The greater number visiting the trap appeared to be due to its being largely blue, rather than being three-dimensional or raised above the ground. Through a series of variations of target design we show that a blue-and-black panel of cloth (0.06 m²) flanked by a panel (0.06 m²) of fine black netting, placed at ground level, would be about ten times more cost-effective than traps or large targets in control campaigns. This finding has important implications for controlling all subspecies of *G. fuscipes*, which are currently responsible for more than 90 percent of sleeping sickness cases.

14983. **Omolo, M. O., Hassanali, A., Mpiana, S., Esterhuizen, J., Lindh, J., Lehane, M. J., Solano, P., Rayaisse, J. B., Vale, G. A., Torr, S. J. & Tirados, I., 2009.** Prospects for developing odour baits to control *Glossina fuscipes* spp., the major vector of human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **3** (5): e435.

International Center for Insect Physiology and Ecology, ICIPE, Nairobi, Kenya.

We are attempting to develop cost-effective control methods for the important vector of sleeping sickness, *Glossina fuscipes* spp. Responses of the tsetse flies *Glossina fuscipes fuscipes* (in Kenya) and *G. f. quanzensis* (in Democratic Republic of Congo) to natural host odours are reported. Arrangements of electric nets were used to assess the effect of cattle-, human- and pig-odour on (1) the numbers of tsetse attracted to the odour source and (2) the proportion of flies that landed on a black target (1x1 m). In addition, responses to monitor lizard (*Varanus niloticus*) were assessed in Kenya. The effects of all four odours on the proportion of tsetse that entered a biconical trap were also determined. Sources of natural host odour were produced by placing live hosts in a tent or metal hut (volumes approximately 16 m³ from which the air was exhausted at approximately 2 000 L/min. Odours from cattle, pigs and humans had no significant effect on attraction of *G. f. fuscipes* but lizard odour doubled the catch (P<0.05). Similarly, mammalian odours had no significant effect on landing or trap entry whereas lizard odour increased these responses significantly: landing responses increased significantly by 22 percent for males and 10 percent for females; the increase in trap efficiency was relatively slight (5-10 percent) and not always significant. For *G. f. quanzensis*, only pig odour had a consistent effect, doubling the catch of females attracted to the source and increasing the landing response for females by approximately 15 percent. Dispensing CO₂ at doses equivalent to natural hosts suggested that the response of *G. f. fuscipes* to lizard odour was not due to CO₂. For *G. f. quanzensis*, pig odour and CO₂ attracted similar numbers of tsetse, but CO₂ had no material effect on the landing response. The results suggest that identifying kairomones present in lizard odour for *G. f. fuscipes* and pig odour for *G. f. quanzensis* may improve the performance of targets for controlling these species.

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

[See also 32: 14962, 15024, 15026, 15027, 15033].

14984. **Allou, K., Acapovi-Yao, G., Kaba, D., Bosson-Vanga, H., Solano, P. & N'Goran, K. E., 2009.** Eco-distribution and *Trypanosoma* infection of *Glossina palpalis palpalis* in the Banco forest of and its relics, Abidjan (Côte d'Ivoire). *Parasite*, **16** (4): 289-295.

Université d'Abidjan-Cocody, Laboratoire de Zoologie, 22 BP 582, Abidjan 22, Côte d'Ivoire.

In order to implement an anti-vector programme in the suburb of Abidjan (Côte d'Ivoire), investigations were conducted to assess tsetse fly densities as well as infection with trypanosomes. Catches were carried out during the rainy season and dry season with Vavoua traps laid during four consecutive days in different sites (Banco forest, Abidjan zoological park, area around the University of Abobo-Adjame). One species of tsetse fly (*Glossina palpalis palpalis*) and two species of trypanosomes (*Trypanosoma congolense*, *T. vivax*) were revealed. The apparent density per trap per day (DAP) was very high in the zoological park, 54.8 tsetse fly/trap/day during the dry season and 28.1 during the rainy season. At the University of Abobo-Adjame, the DAP was respectively 13.5 and 8.1 tsetse fly/trap/day during the rainy and dry seasons, and in the wet and dry seasons it was only 0.9 and 0.8 in the Banco Forest. The physiological age on all sites was as follows: 57.5 percent of old parous, 39 percent of young parous and 3.6 percent of nulliparous in the rainy season. These proportions varied from 51.9 percent for young parous, 47.1 percent for old parous and 1 percent for nulliparous in the dry season. The overall infection rate was estimated at 20.7 percent in the rainy season and 20 percent in the dry season. Statistical analysis showed a significant difference in the distribution of infection rates.

14985. **Averis, S., Thompson, R. C., Lymbery, A. J., Wayne, A. F., Morris, K. D. & Smith, A., 2009.** The diversity, distribution and host-parasite associations of trypanosomes in Western Australian wildlife. *Parasitology*, **136** (11): 1269-1279.

WHO Collaborating Centre for the Molecular Epidemiology of Parasitic Infections, State Agricultural Biotechnology Centre, School of Veterinary and Biomedical Sciences, Murdoch University, South Street, Western Australia 6150, Australia.

Little is known regarding the diversity, distribution or host-parasite associations of *Trypanosoma* spp. in Australian wildlife. Here we report on an investigation based on divergence of the 18S rRNA gene of trypanosomes isolated from a range of hosts and varied geographical locations. A total of 371 individuals representing 19 species of native animals from 14 different locations were screened. In total, 32 individuals from 9 different species tested positive for the parasite. Phylogenetic analysis revealed considerable parasite diversity with no clear geographical distribution and no evidence of host specificity. In general, it appears that Australian *Trypanosoma* spp. are widespread, with several genotypes appearing in multiple host species and in varied locations including both mainland areas and offshore

islands. Some host species were found to be susceptible to multiple genotypes, but no individuals were infected with more than a single isolate.

14986. **Balmer, O., Stearns, S. C., Schotzau, A. & Brun, R., 2009.** Intraspecific competition between co-infecting parasite strains enhances host survival in African trypanosomes. *Ecology*, **90** (12): 3367-3378.

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

It is becoming increasingly clear that under natural conditions parasitic infections commonly consist of co-infections with multiple conspecific strains. Multiple-strain infections lead to intraspecific interactions and may have important ecological and evolutionary effects on both hosts and parasites. However, experimental evidence on intraspecific competition or facilitation in infections has been scarce because of the technical challenges of distinguishing and tracking individual co-infecting strains. To overcome this limitation, we engineered transgenic strains of the protozoan parasite *Trypanosoma brucei*, the causal agent of human African sleeping sickness. Different strains were transfected with fluorescence genes of different colours to make them visually distinguishable in order to investigate the effects of multiple-strain infections on parasite population dynamics and host fitness. We infected mice either with each strain alone or with mixes of two strains. Our results show a strong mutual competitive suppression of co-infecting *T. brucei* strains very early in infection. This mutual suppression changes within-host parasite dynamics and alleviates the effects of infection on the host. The strength of suppression depends on the density of the co-infecting strain, and differences in life-history traits between the strains determine the consequences of strain-strain competition for the host. Unexpectedly, co-infection with a less virulent strain significantly enhances host survival (+15 percent). Analysis of the strain dynamics reveals that this is due to the suppression of the density of the more virulent strain (-33 percent), whose degree of impact ultimately determines the physical condition of the host. The competitive suppression is likely caused by allelopathic interference or by apparent competition mediated by strain-specific immune responses. These findings highlight the importance of intraspecific variation for parasite-parasite and parasite-host interactions. To fully understand parasite and disease dynamics, the genetic diversity of infections must be taken into account. Through changes in parasite dynamics, intraspecific variation may further affect transmission dynamics and select for increased virulence of each strain. The precise mechanisms underlying mutual suppression are not yet understood but may be exploitable to fight this devastating parasite. Our results are therefore not only of basic ecological interest investigating an important form of intraspecific competition, but may also have applied relevance for public health.

14987. **Batchelor, N. A., Atkinson, P. M., Gething, P. W., Picozzi, K., Fevre, E. M., Kakembo, A. S. & Welburn, S. C., 2009.** Spatial predictions of Rhodesian Human African Trypanosomiasis (sleeping sickness) prevalence in Kaberamaido and Dokolo, two newly affected districts of Uganda. *PLoS Neglected Tropical Diseases*, **3** (12): e563.

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

The continued northwards spread of Rhodesian sleeping sickness or Human African Trypanosomosis (HAT) within Uganda is raising concerns of overlap with the Gambian form of the disease. Disease convergence would result in compromised diagnosis and treatment for HAT. Spatial determinants for HAT are poorly understood across small areas. This study examines the relationships between Rhodesian HAT and several environmental, climatic and social factors in two newly affected districts, Kaberamaido and Dokolo. A one-step logistic regression analysis of HAT prevalence and a two-step logistic regression method permitted separate analysis of both HAT occurrence and HAT prevalence. Both the occurrence and prevalence of HAT were negatively correlated with distance to the closest livestock market in all models. The significance of distance to the closest livestock market strongly indicates that HAT may have been introduced to this previously unaffected area via the movement of infected, untreated livestock from endemic areas. This illustrates the importance of the animal reservoir in disease transmission, and highlights the need for trypanosomosis control in livestock and the stringent implementation of regulations requiring the treatment of cattle prior to sale at livestock markets to prevent any further spread of Rhodesian HAT within Uganda.

14988. **Bouyer, J., Balenghien, T., Ravel, S., Vial, L., Sidibe, I., Thevenon, S., Solano, P. & De Meeus, T., 2009.** Population sizes and dispersal pattern of tsetse flies: rolling on the river? *Molecular Ecology*, **18** (13): 2787-2797.

Cirad, UMR CIRAD-INRA Contrôle des maladies animales, Campus International de Baillarguet, F34398, Montpellier, France. [bouyer@cirad.fr].

The West African trypanosomoses are mostly transmitted by riverine species of tsetse fly. In this study, we estimate the dispersal and population size of tsetse populations located along the Mouhoun river in Burkina Faso where tsetse habitats are experiencing increasing fragmentation caused by human encroachment. Dispersal estimated through direct (mark and recapture) and indirect (genetic isolation by distance) methods appeared consistent with one another. In these fragmented landscapes, tsetse flies displayed localized, small subpopulations with relatively short effective dispersal. We discuss how such information is crucial for designing optimal strategies for eliminating this threat. To estimate ecological parameters of wild animal populations, the genetic measures are both a cost- and time-effective alternative to mark-release-recapture. They can be applied to other vector-borne diseases of medical and/or economic importance.

14989. **DeVisser, M. H. & Messina, J. P., 2009.** Optimum land cover products for use in a *Glossina-morsitans* habitat model of Kenya. *International Journal of Health Geographics*, **8**: 39.

Department of Geography and Center for Global Change and Earth Observations, Michigan State University, East Lansing, MI, USA. [devisse6@msu.edu].

Tsetse flies are the primary vector for African trypanosomosis, a disease that affects both humans and livestock across the continent of Africa. In 1973 tsetse flies were estimated to inhabit 22 percent of Kenya; by 1996 that number had risen to roughly 34 percent. Efforts to control the disease were hampered by a lack of information and costs associated with the identification of infested areas. Given changing spatial and demographic factors, a model that can predict suitable tsetse fly habitat based on land cover and climate change is critical to efforts aimed at controlling the disease. In this paper we present a generalizable method, using a modified Mapcurves goodness of fit test, to evaluate the existing publicly available land cover products to determine which products perform the best at identifying suitable tsetse fly land cover. For single date applications, Africover was determined to be the best land use land cover (LULC) product for tsetse modeling. However, for changing habitats, whether climatically or anthropogenically forced, the IGBP DISCover and MODIS type 1 products were determined to be most practical. It is concluded that the method can be used to differentiate between various LULC products and be applied to any such research when there is a known relationship between a species and land cover.

14990. **Ducheyne, E., Mweempwa, C., De Pus, C., Vernieuwe, H., De Deken, R., Hendrickx, G. & Van den Bossche, P., 2009.** The impact of habitat fragmentation on tsetse abundance on the plateau of eastern Zambia. *Preventive Veterinary Medicine*, **91** (1): 11-18.

Avia-GIS, Risschotlei 33, 2980 Zoersel, Belgium. [educheyne@avia-gis.be].

Tsetse-transmitted human or livestock trypanosomosis is one of the major constraints to rural development in sub-Saharan Africa. The epidemiology of the disease is determined largely by tsetse fly density. A major factor contributing to tsetse population density is the availability of suitable habitat. In large parts of Africa, encroachment of people and their livestock resulted in a destruction and fragmentation of such suitable habitat. To determine the effect of habitat change on tsetse density a study was initiated in a tsetse-infested zone of eastern Zambia. The study area represents a gradient of habitat change, starting from a zone with high levels of habitat destruction and ending in an area where livestock and people are almost absent. To determine the distribution and density of the fly, tsetse surveys were conducted throughout the study area in the dry and in the rainy season. Landsat ETM+ imagery covering the study area was classified into four land cover classes (munga, miombo, agriculture and settlements) and two auxiliary spectral classes (clouds and shadow) using a Gaussian Maximum Likelihood Classifier. The classes were regrouped into natural vegetation and agricultural zone. The binary images were overlaid with hexagons to obtain the spatial spectrum of spatial pattern. Hexagonal coverage was selected because of its compact and regular form. To identify scale-specific spatial patterns and associated entomological phenomena, the size of the hexagonal coverage was varied (250 and 500 m). Coverage, total class area, mean patch size, number of patches and patch size standard deviation were used as fragmentation indices. Based on the fragmentation index values, the study zone was classified using a Partitioning Around Medoids (PAM) method. The number of classes was determined using the Wilks' lambda coefficient. To determine the impact of habitat fragmentation on tsetse abundance, the correlation between the fragmentation indices and the index of apparent density of the flies was determined and habitat changes most affecting tsetse abundance were identified. From this it followed that there is a clear relationship between habitat fragmentation and the abundance of tsetse flies. Heavily

fragmented areas have lower numbers of tsetse flies, but when the fragmentation of natural vegetation decreases, the number of tsetse flies increases following a sigmoidal-like curve.

14991. **Gondwe, N., Marcotty, T., Vanwambeke, S. O., De Pus, C., Mulumba, M. & Van den Bossche, P., 2009.** Distribution and density of tsetse flies (*Glossinidae*: Diptera) at the game/people/livestock interface of the Nkhotakota Game Reserve human sleeping sickness focus in Malawi. *Ecohealth*, **6** (2): 260-265.

Centre for Ticks and Tick-Borne Diseases, Lilongwe, Malawi.

In large parts sub-Saharan Africa, tsetse flies, the vectors of African human or animal trypanosomosis, are, or will in the foreseeable future, be confined to protected areas such as game or national parks. Challenge of people and livestock is likely to occur at the game/livestock/people interface of such infested areas. Since tsetse control in protected areas is difficult, management of trypanosomosis in people and/or livestock requires a good understanding of tsetse population dynamics along such interfaces. The Nkhotakota Game Reserve, an important focus of human trypanosomosis in Malawi, is a tsetse-infested protected area surrounded by a virtually tsetse-free zone. The abundance of tsetse (*Glossina morsitans morsitans*) along the interface, within and outside the game reserve, was monitored over 15 months using epsilon traps. A land cover map described the vegetation surrounding the traps. Few flies were captured outside the reserve. Inside, the abundance of tsetse at the interface was low but increased away from the boundary. This uneven distribution of tsetse inside the reserve is attributed to the uneven distribution of wildlife, the main host of tsetse, being concentrated deeper inside the reserve. Challenge of people and livestock at the interface is thus expected to be low, and cases of trypanosomosis are likely due to people and/or livestock entering the reserve. Effective control of trypanosomosis in people and livestock could be achieved by increasing the awareness among people of dangers associated with entering the reserve.

14992. **Harraca, V., Syed, Z. & Guerin, P. M., 2009.** Olfactory and behavioural responses of tsetse flies, *Glossina* spp., to rumen metabolites. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology*, **195** (9): 815-824.

Faculty of Science, Institute of Biology, University of Neuchatel, Neuchatel, Switzerland. [vincent.harraca@ekol.lu.se].

Herbivores provide tsetse flies with a blood meal, and both wild and domesticated ruminants dominate as hosts. As volatile metabolites from the rumen are regularly eructed with rumen gas, these products could serve tsetse flies during host searching. To test this, we first established that the odour of rumen fluid is attractive to hungry *Glossina pallidipes* in a wind tunnel. We then made antennogram recordings from three tsetse species (*G. pallidipes morsitans* group, *G. fuscipes palpalis* group and *G. brevipalpis fusca* group) coupled to gas chromatographic analysis of rumen fluid odour and of its acidic, mildly acidic and neutral fractions. This shows tsetse flies can detect terpenes, ketones, carboxylic acids, aliphatic aldehydes, sulphides, phenols and indoles from this biological substrate. A mixture of carboxylic acids at a ratio similar to that present in rumen fluid induced behavioural responses from *G. pallidipes* in the wind tunnel that were moderately better than the solvent

control. The similarities in the sensory responses of the tsetse fly species to metabolites from ruminants demonstrated in this study testify to a contribution of habitat exploitation by these vertebrates in the Africa-wide distribution of tsetse.

14993. **Janelle, J., Koffi, M., Jamonneau, V., Patrel, D., Cuny, G. & Ravel, S., 2009.** Monitoring the pleomorphism of *Trypanosoma brucei gambiense* isolates in mouse: impact on its transmissibility to *Glossina palpalis gambiensis*. *Infection, Genetics and Evolution*, **9** (6): 1260-1264.

CIRAD, UMR Trypanosomes, Montpellier F-34398, France.

Substantial differences have been observed between the cyclical transmission of three *Trypanosoma brucei gambiense* field isolates in *Glossina palpalis gambiensis*. Differences in the pleomorphism of these isolates in rodent used to provide the infective feed to *Glossina*, could explain such results, since stumpy forms are preadapted for differentiation to procyclic forms when taken up in a tsetse blood meal. To assess this possibility, mice were immunosuppressed and inoculated intraperitoneally with the three isolates (six mice for each trypanosome isolate); parasitaemia and pleomorphism were then determined daily for each mouse. The three *T. b. gambiense* isolates induced different infection patterns in mouse. The parasitaemia peak was rapidly reached for all the isolates and maintained until mice death for two isolates, while the third isolate rapidly showed a falling phase followed by a second parasitaemia plateau. The proportion of the stumpy forms varied from 15 percent to 70 percent over the duration of the experiment and according to the isolate. One isolate, which displayed the highest proportion of stumpy forms and reached the stumpy peak at the onset of the falling phase of parasitaemia, was used to study the relationship between the proportion of stumpy forms and transmissibility to tsetse fly. The results indicated that the transmissibility of trypanosomes was not correlated to the proportion of non-dividing stumpy forms.

14994. **Madanitsa, M., Chisi, J. & Ngwira, B., 2009.** The epidemiology of trypanosomiasis in Rumphi district, Malawi: a ten year retrospective study. *Malawi Medical Journal*, **21** (1): 22-27.

College of Medicine, Malawi. [mmdanitsa2004@yahoo.com].

Human African trypanosomosis (HAT) is caused by two species of the tsetse fly vectored protozoan hemoflagellates belonging to *Trypanosoma brucei*, namely *T. b. gambiense* which predominates in Western Africa and follows a chronic disease course and *T. b. rhodensiense* which is more prevalent in Southern and Eastern Africa, Malawi included, and follows a more acute and aggressive disease course. Previous studies in the Democratic Republic of Congo, Angola, Uganda and Sudan have demonstrated that the prevalence rates of *T. b. rhodensiense* infection have reached epidemic proportions. This study had the objective of describing the epidemiology of trypanosomosis in Rumphi District over the past ten years. A total of 163 records from January 2000 to December 2006 were retrospectively studied. There were more male than female cases (121 vs. 40) within the 20 - 29 years age bracket which had the highest number of cases (26.3 percent, n = 160). Stage 2 HAT was the commonest stage at presentation (58.2 percent, n = 158) with the patients in the same being 3.5 times more likely to die than those with stage 1 HAT. Case fatality rates for late and early

stage disease were 21.5 percent (n = 92) and 7.2 percent (n = 66) respectively with 84.6 percent having been cured (n = 162). Convulsions were associated with fatal disease outcome and the majority of cases (97.2 percent, n = 103) lived within 5 km of the Vwaza game reserve boundary. It is concluded that more men have been infected than women, with a high involvement in the 20 - 29 age brackets. A dramatic increase with active case finding indicates a high under-detection of the disease with late stage HAT being predominant at presentation. Though it has been found that cases with late stage disease have an increased likelihood of dying compared with those in early stage HAT, the high proportion of successful treatment indicates that the disease still carries a high degree of favourable outcome with treatment. It has also been demonstrated in this study that more than 95 percent of trypanosomosis cases live within 5 km of a game reserve boundary. Disease interventions should be implemented in areas within 5 km of marshland game reserve boundaries as priority areas.

14995. **Massusi, J. A., Djieto-Lordon, C., Njiokou, F., Laveissiere, C. & van der Ploeg, J. D., 2009.** Influence of habitat and seasonal variation on wild mammal diversity and distribution with special reference to the *Trypanosoma brucei gambiense* host-reservoir in Bipindi (Cameroon). *Acta Tropica*, **112** (3): 308-315.

Institute of Agricultural Research for Development, P.O. Box 167, Meyomessala, Cameroon. [massuja@gmail.com].

To evaluate the role of wildlife in the resurgence and perenisation of human African trypanosomosis (HAT), we investigated the influence of habitat and seasonal variations on the diversity and spatial distribution of wild mammals, with special reference to those recognized as potential host-reservoirs of *Trypanosoma brucei gambiense* in Bipindi (southwestern Cameroon). To achieve this, we carried out transect surveys in four habitat types over two years. A total of 31 mammal species were recorded, of which 14 occurred in the undisturbed forest, 9 in cocoa plantations, 11 in farmlands and 11 in village-adjacent gallery forests. Among them, six species (*Cephalophus monticola*, *Cephalophus dorsalis*, *Atherurus africanus*, *Cricetomys emini*, *Nandinia binotata* and *Cercopithecus nictitans*), known as reservoir hosts of *T. b. gambiense*, occurred in all kinds of habitats suitable or unsuited to *Glossina palpalis palpalis* and in all seasons. These species are the most involved in the transmission cycle (human being/tsetse flies/wild animals). *Cercopithecus cephus*, *Miopithecus talapoin* and *Heliosciurus rufobrachium* host *Trypanosoma brucei* spp.; however, only *C. cephus* does not occur permanently in the suitable habitat of *G. palpalis palpalis*. In general, some species (*C. monticola*, *Tragelaphus spekei* and *C. emini*) showed a slight density increase from the long dry to the heavy rainy season within the undisturbed and farmland habitats, and a slight decrease within cocoa plantations and village-adjacent forests in the same period. The density of *A. africanus* increased greatly from the long dry season to the heavy rainy season in the undisturbed forest while the density of primates in this habitat decreased slightly from the long dry season to the heavy rainy season. These variations indicate a permanent movement of wild mammal reservoirs or feeding hosts from one biotope to another over the seasons. *Thryonomys swinderianus* needs to be investigated because it occurs permanently in the suitable habitat of *G. palpalis palpalis* and *Potamochoerus porcus* for its genetic similarities to domestic pigs, favourable feeding hosts

14996. **Mbida Mbida, J. A., Mimpfoundi, R., Njiokou, F., Manga, L. & Laveissiere, C., 2009.** Distribution and ecology of the savannah human African trypanosomiasis vectors in disturbed forest zone in south Cameroon: about case in the Doume forest. *Bulletin of the Exotic Pathology Society*, **102** (2): 101-105.

Laboratoire de biologie générale, Faculté des sciences BP 812, Yaoundé, Cameroun. [mbidajean@yahoo.fr].

Vector control through trapping in the foci of humid forest areas is rather difficult because of the wide spread of tsetse flies and transmission sites of human African trypanosomosis. In fact, traps should be *a priori* set up everywhere to stop the transmission. The identification of the disease transmission sites enables efficient trapping through localization of dangerous tsetse flies habitats needing vector control measures. The study of adult tsetse flies and teneral tsetse flies spatial distribution and human vector contacts was conducted in Doume to determine the transmission of human African trypanosomosis for efficient vector control. *Glossina fuscipes fuscipes* was the only tsetse fly captured with a very low apparent density of 0.13 tsetse flies/trap/day. Furthermore, disease transmission in the focus was not found uniform. In fact, human vector contacts were high in two villages (Paki and Mendin) located in the highly disturbed forest zones. These contacts occur in humid shallows where teneral tsetse flies were only captured around streams and forest galleries. The Doume focus therefore presents the characteristics of savannah focus where river banks and nearby biotopes are the main target sites for vector control campaigns.

14997. **Peacock, L., Ferris, V., Bailey, M. & Gibson, W., 2009.** Intracloal mating occurs during tsetse transmission of *Trypanosoma brucei*. *Parasite Vectors*, **2** (1): 43.

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

Mating in *Trypanosoma brucei* is a non-obligatory event, triggered by the co-occurrence of different strains in the salivary glands of the vector. Recombinants that result from intra- rather than interclonal mating have been detected, but only in crosses of two different trypanosome strains. This has led to the hypothesis that when trypanosomes recognize a different strain, they release a diffusible factor or pheromone that triggers mating in any cell in the vicinity whether it is of the same or a different strain. This idea assumes that the trypanosome can recognize self and non-self, although there is as yet no evidence for the existence of mating types in *T. brucei*. We investigated intracloal mating in *T. b. brucei* by crossing red and green fluorescent lines of a single strain, so that recombinant progeny can be detected in the fly by yellow fluorescence. For strain 1738, seven flies had both red and green trypanosomes in the salivary glands and, in three, yellow trypanosomes were also observed, although they could not be recovered for subsequent analysis. Nonetheless, both red and non-fluorescent clones from these flies had recombinant genotypes as judged by microsatellite and karyotype analyses, and some also had raised DNA contents, suggesting recombination or genome duplication. Strain J10 produced similar results indicative of intracloal mating. In contrast, trypanosome clones recovered from other flies showed that genotypes can be transmitted with fidelity. When a yellow hybrid clone expressing both red and green fluorescent protein genes was transmitted, the salivary glands contained a mixture of fluorescent-coloured trypanosomes, but only yellow and red clones were recovered. While

loss of the GFP gene in the red clones could have resulted from gene conversion, some of these clones showed loss of heterozygosity and raised DNA contents as in the other single strain transmissions. Our observations suggest that many recombinants are non-viable after intraclonal mating. In conclusion, we have demonstrated intraclonal mating during fly transmission of *T. b. brucei*, contrary to previous findings that recombination occurs only when another strain is present. It is thus no longer possible to assume that *T. b. brucei* remains genetically unaltered after fly transmission.

14998. **Rademaker, V., Herrera, H. M., Raffel, T. R., D'Andrea, P. S., Freitas, T. P., Abreu, U. G., Hudson, P. J. & Jansen, A. M., 2009.** What is the role of small rodents in the transmission cycle of *Trypanosoma cruzi* and *Trypanosoma evansi* (Kinetoplastida Trypanosomatidae)? A study case in the Brazilian Pantanal. *Acta Tropica*, **111** (2): 102-107.

Center for Infectious Disease Dynamics, The Pennsylvania State University, Biology Department, 208 Mueller Lab, University Park, PA 16802-5301, USA. [vrademaker@yahoo.com].

Determining the reservoir hosts for parasites is crucial for designing control measures, but it is often difficult to identify the role that each host species plays in maintaining the cycle of infection in the wild. One way to identify potential maintenance hosts is to estimate key parameters associated with transmission and pathogenicity. Here we assess the potential for three native rodent species of the Brazilian Pantanal (*Clyomys laticeps*, *Thrichomys pachyurus* and *Oecomys mamorae*) to act as reservoir or maintenance hosts of *Trypanosoma evansi*, an important parasite of domestic livestock. By analyzing blood parameters of naturally infected wild-caught rodents of these species, we compared their levels of parasitaemia and anaemia due to *T. evansi* infection with literature values for other host species infected by this parasite. We also analyzed levels of these blood parameters relative to infection by *Trypanosoma cruzi*, the causative agent of Chagas disease in humans, for which wild rodents are already thought to be important reservoir species. All three species showed low impacts of the two trypanosomes on their blood parameters compared to other species, suggesting that they experience a low virulence of trypanosome infection under natural conditions in the Pantanal and might act as maintenance hosts of trypanosome infections. The low parasitaemia of trypanosome infections suggests that these rodents play a secondary role in the transmission cycle compared with other species, especially compared with the capybara (*Hydrochaeris hydrochaeris*) which also experiences low pathogenicity due to infection despite much higher levels of parasitaemia.

14999. **Sharma, R., Gluenz, E., Peacock, L., Gibson, W., Gull, K. & Carrington, M., 2009.** The heart of darkness: growth and form of *Trypanosoma brucei* in the tsetse fly. *Trends in Parasitology*, **25** (11): 517-524.

Department of Biochemistry, University of Cambridge, 80 Tennis Court Road, Cambridge CB2 1GA, UK. [reuben@vet.upm.edu.my].

The first description of African trypanosomes was made over a century ago. The importance of the tsetse in transmission and cyclic development of trypanosomes was discovered soon afterwards, and has been the focus of numerous studies since. However,

investigation of trypanosomes in tsetse flies requires high resource investment and unusual patience; hence, many facets of trypanosome biology in the tsetse remain to be characterized despite the long history of research. Here, current knowledge and questions about some of the developmental changes in trypanosomes that occur in tsetse flies are summarized, along with recent technical advances that can now be used to provide some answers.

15000. **Tshimungu, K., Okenge, L. N., Mukeba, J. N., Kande, V. B. & De Mol, P., 2009.** Epidemiological, clinical and sociodemographic characteristics of human African trypanosomiasis (HAT) in and around Kinshasa, Democratic Republic of Congo. *Santé*, **19** (2): 73-80.

Laboratoire de microbiologie médicale, Université de Liège, CHU Sart-Tilman (B23), 4000 Liège, Belgium. [ftshimungu@yahoo.fr].

Despite efforts to control human African trypanosomiasis (HAT) in the field, this infection remains prevalent in endemic or epidemic form in most of its traditional habitats. In the Democratic Republic of Congo (DRC), HAT has extended beyond rural areas to reach large cities such as Kinshasa. The objective of this study was to analyse the characteristics of trypanosomiasis patients (cases) in Kinshasa and to compare them to those of healthy controls. This case-control study allowed us to compare case patients and controls for some epidemiologic, clinical and sociodemographic characteristics. In all, 1 764 people (588 case-patients and 1 176 controls) were interviewed according to a structured questionnaire. Case-patients were infected with trypanosomiasis and entered the National Human African Trypanosomiasis Program (PNLTHA-DRC) from January 2004 through December 2005. Controls were matched for sex, age and residence to the corresponding case-patient, but had negative results from the Card Agglutination Trypanosomiasis Test (CATT-Test) whole-blood serologic analysis. Each patient was matched with two controls. Cases were identified in all 24 districts of Kinshasa, but were concentrated in the outskirts (outlying areas and southern expansion) and in rural areas. Overall, 25 percent (144/588) of case-patients lived in urbanized areas. People in the labour market (aged 20-49 years) were affected more often than others. HAT affected men and women equally. It also affected at higher rates people who moved around a lot and those who worked in rural or domestic activities, especially those in close contact with watercourses. Sleep disorders were the primary clinical sign (85 percent). Cervical adenopathies were observed frequently (66 percent). Fever was reported in 68 percent of case-patients. Most (73.5 percent) were diagnosed at a very advanced stage of infection (meningoencephalitic or neurological stage). These results highlight several modifiable or avoidable characteristics associated with HAT. Interventions on them might make it possible to reduce the morbidity and mortality rates associated with HAT and prevent wider extension of this disease.

15001. **Wang, J., Wu, Y., Yang, G. & Aksoy, S., 2009.** Interactions between mutualist *Wigglesworthia* and tsetse peptidoglycan recognition protein (PGRP-LB) influence trypanosome transmission. *Proceedings of the National Academy of Sciences USA*, **106** (29): 12133-12138.

Department of Epidemiology of Microbial Diseases, Yale School of Public Health, New Haven, CT 06520, USA.

Tsetse flies, the sole vectors of African trypanosomes, have coevolved with mutualistic endosymbiont *Wigglesworthia glossinidia*. Elimination of *Wigglesworthia* renders tsetse sterile and increases their trypanosome infection susceptibility. We show that a tsetse peptidoglycan recognition protein (PGRP-LB) is crucial for symbiotic tolerance and trypanosome infection processes. Tsetse *pgrp-lb* is expressed in the *Wigglesworthia*-harboring organ (bacteriome) in the midgut, and its level of expression correlates with symbiont numbers. Adult tsetse cured of *Wigglesworthia* infections have significantly lower *pgrp-lb* levels than corresponding normal adults. RNA interference (RNAi)-mediated depletion of *pgrp-lb* results in the activation of the immune deficiency (IMD) signaling pathway and leads to the synthesis of antimicrobial peptides (AMPs), which decrease *Wigglesworthia* density. Depletion of *pgrp-lb* also increases the host's susceptibility to trypanosome infections. Finally, parasitized adults have significantly lower *pgrp-lb* levels than flies, which have successfully eliminated trypanosome infections. When both PGRP-LB and IMD immunity pathway functions are blocked, flies become unusually susceptible to parasitism. Based on the presence of conserved amidase domains, tsetse PGRP-LB may scavenge the peptidoglycan (PGN) released by *Wigglesworthia* and prevent the activation of symbiont-damaging host immune responses. In addition, tsetse PGRP-LB may have an anti-protozoal activity that confers parasite resistance. The symbiotic adaptations and the limited exposure of tsetse to foreign microbes may have led to the considerable differences in *pgrp-lb* expression and regulation noted in tsetse from that of closely related *Drosophila*. A dynamic interplay between *Wigglesworthia* and host immunity apparently is influential in tsetse's ability to transmit trypanosomes.

5. HUMAN TRYPANOSOMOSIS

(a) SURVEILLANCE

15002. **Bouteille, B., Mpandzou, G., Cespuglio, R., Ngampo, S., Peeling, R. W., Vincendeau, P. & Buguet, A., 2009.** Cerebrospinal fluid B lymphocyte identification for diagnosis and follow-up in human African trypanosomiasis in the field. *Tropical Medicine and International Health*. Oct 5. **e Publication ahead of print.**

Laboratory of Parasitology, EA 3174, Faculty of Medicine, University of Limoges, France.

In human African trypanosomosis (HAT, sleeping sickness), staging of disease and treatment follow-up relies on the white cell count in the cerebrospinal fluid (CSF). As B lymphocytes (CD19 positive cells) are not found in the CSF of healthy individuals but occur in neurological disorders such as multiple sclerosis, the B lymphocyte count may be useful for field diagnosis/staging and therapeutic follow-up in HAT. Seventy-one HAT patients were diagnosed and 50 were followed-up 6-24 months after treatment. White cell counts were used for conventional staging (stage 1, ≤ 5 cells/ μ l CSF, n = 42; stage 2, ≥ 20 cells/ μ l, n = 16) and intermediate stage (6-19 cells/ μ l, n = 13). Slides containing 1 μ l of CSF mixed with Dynabeads® CD19 pan B were examined microscopically to detect B cell rosettes (bound to at least four beads). Stage 1 patients exhibited zero (n = 37) or one CSF rosette/ μ l (n = 5), contrary to most stage 2 patients (14/16: ≥ 2 rosettes/ μ l). Intermediate stage patients

expressed 0 (n = 9), 1 (n = 3) or 2 (n = 1) rosettes/ μ l of CSF. During follow-up, rosette counts correlated with white cell count staging but were much easier to read. It is concluded that B cell rosettes being easily detected in the CSF in field conditions may be proposed to replace white cell count for defining HAT stages 1 and 2 and limit uncertainty in treatment decision in patients with the intermediate stage.

15003. **Cecchi, G., Courtin, F., Paone, M., Diarra, A., Franco, J. R., Mattioli, R. C. & Simarro, P. P., 2009.** Mapping sleeping sickness in Western Africa in a context of demographic transition and climate change. *Parasite*, **16** (2): 99-106.

Food and Agriculture Organization of the United Nations (FAO), Animal Production and Health Division, Viale delle Terme di Caracalla, 1, 00153, Rome, Italy.

Human population growth, climate change and economic development are causing major environmental modifications in Western Africa, which will have important repercussions on the epidemiology of sleeping sickness. A new initiative, the Atlas of Human African trypanosomosis (HAT), aims at assembling and geo-referencing all epidemiological data derived from both active screening activities and passive surveillance. A geographic database enables up-to-date disease maps to be generated at a range of scales and of unprecedented spatial accuracy. We present preliminary results for seven West African countries (Benin, Burkina Faso, Côte d'Ivoire, Ghana, Guinea, Mali and Togo) and briefly discuss the relevance of the Atlas for future monitoring, control and research activities.

15004. **Gautret, P., Clerinx, J., Caumes, E., Simon, F., Jensenius, M., Loutan, L., Schlagenhauf, P., Castelli, F., Freedman, D., Miller, A., Bronner, U. & Parola, P., 2009.** Imported human African trypanosomiasis in Europe, 2005-2009. *Eurosurveillance*, **14** (36).

Infectious and Tropical Disease Unit, Hôpital Nord AP-HM, Marseille, France. [surveillance@eurotravnet.eu].

Physicians in Europe are likely to see more African trypanosomosis cases because of the increasing popularity of travel to Africa. In this paper the literature on imported cases in Europe since 2005 is reviewed. Because of the high mortality risk associated with acute Rhodesian trypanosomosis, travellers should be informed about preventive measures and the early disease manifestations.

15005. **Hasker, E., Mitashi, P., Baelmans, R., Lutumba, P., Jacquet, D., Lejon, V., Kande, V., Declercq, J., Van der Veken, W. & Boelaert, M., 2009.** A new format of the CATT test for the detection of Human African Trypanosomiasis, designed for use in peripheral health facilities. *Tropical Medicine and International Health*. **e Publication ahead of print.**

Department of Public Health, Epidemiology and Disease Control Unit, Institute of Tropical Medicine, Antwerp, Belgium.

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

15006. **Louis, F. J., Djimadoum, N. A., Kohagne, T. L. & Simarro, P. P., 2009.** The Mandoul human African trypanosomiasis focus in Chad: from evaluation to control. *Médecine Tropicale (Mars)*, **69** (1): 7-12.

Programme sous-régional de lutte contre la trypanosomiase humaine africaine (PSR-THA) de l'OCEAC, Yaoundé, Cameroun. [louis_oceac@yahoo.fr].

The Mandoul focus of human African trypanosomiasis in southern Chad was first described by Gaston Muraz in the 1920s. After 40 years of control measures, case reports became rare and the focus was forgotten. However, the number of cases began to increase in 1993 and coordinated control measures were implemented in 2002. The first phase of control consisted of mapping out the focus that was shown to involve 45 villages and camps on both sides of the Mandoul River. The estimated number of inhabitants in the area is 20 000 and the endemic prevalence was 3.78 percent. Dynamic passive screening and regular active screening undertaken in the framework of the Chadian human African trypanosomiasis control programme with the assistance of expert technicians from the subregion reduced the prevalence to 0.77 percent in 2006. Although this reduction is encouraging, control measures must be maintained and greater involvement of the health care system will be needed to achieve sustainable control of the disease and ultimately to eliminate human African trypanosomiasis as a public health problem.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **32**: 14965, 15051, 15052, 15053, 15059, 15063].

15007. **Amin, D. N., Rottenberg, M. E., Thomsen, A. R., Mumba, D., Fenger, C., Kristensson, K., Buscher, P., Finsen, B. & Masocha, W., 2009.** Expression and role of CXCL10 during the encephalitic stage of experimental and clinical African trypanosomiasis. *Journal of Infectious Diseases*, **200** (10): 1556-1565.

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden.

Human African trypanosomosis, caused by *Trypanosoma brucei*, involves an early haemolymphatic stage followed by a late encephalitic stage. We studied the expression of chemokines using microarray and enzyme-linked immunosorbent assays in *T. brucei brucei*-infected mice and in patients with human African trypanosomosis and examined their role in controlling brain accumulation of T cells and parasites. Results showed that the messenger RNAs (mRNAs) encoding CXCR3 ligands CXCL9 and CXCL10 demonstrated the greatest increases among chemokines in brain specimens of infected mice, as determined by microarray. CXCL9 and CXCL10 mRNA accumulation was interferon (IFN)-gamma-dependent. Expression of CXCL10 was predominantly observed in astrocytes. Weight loss was registered in wild-type but not in CXCL10(-/-) and CXCR3(-/-) infected mice. Infected CXCL10(-/-) or CXCR3(-/-) mice demonstrated reduced accumulation of trypanosomes and T cells in the brain parenchyma but similar parasitaemia levels, compared with wild-type mice. CXCL10 and IFN-gamma levels were increased in the cerebrospinal fluid of patients with late stage but not early stage human African trypanosomosis. Levels of CXCL10 in patients with late stage human African trypanosomosis were associated with somnolence, low body weight, and trypanosomes in the cerebrospinal fluid. It is concluded that IFN-gamma-dependent CXCL10 is critical for accumulation of T cells and trypanosomes in the brain during experimental African trypanosomosis. Data suggest CXCL10 as a candidate marker for late stage human African trypanosomosis.

15008. **Boda, C., Courtioux, B., Roques, P., Pervieux, L., Vatunga, G., Josenando, T., Ayenengoye, C. R., Bouteille, B., Jauberteau, M. O. & Bisser, S., 2009.** Immunophenotypic lymphocyte profiles in human African trypanosomiasis. *PLoS One*, **4** (7): e6184.

Université de Limoges, IFR 145 GEIST, Institut de Neurologie Tropicale, EA 3174 NeuroEpidémiologie Tropicale et Comparée, Faculté de Médecine, Limoges, France.

Human African trypanosomosis (HAT) is a deadly vector-borne disease caused by an extracellular parasite, the trypanosome. Little is known about the cellular immune responses elicited by this parasite in humans. We used multiparameter flow cytometry to characterize leukocyte immunophenotypes in the blood and cerebrospinal fluid (CSF) of 33 HAT patients and 27 healthy controls identified during a screening campaign in Angola and Gabon. We evaluated the subsets and activation markers of B and T lymphocytes. Patients had a higher percentage of CD19+ B lymphocytes and activated B lymphocytes in the blood than did controls, but lacked activated CD4+ T lymphocytes (CD25+). Patients displayed no increase in the percentage of activated CD8+ T cells (HLA-DR+, CD69+ or CD25+), but memory CD8 T-cell levels (CD8+CD45RA2) were significantly lower in patients than in controls, as were effector CD8 T-cell levels (CD8+CD45RA+CD62L2). No relationship was found between these blood immunophenotypes and disease severity (stage 1 vs 2). However, CD19+ B-cell levels in the CSF increased with disease severity. The patterns of T and B cell activation in HAT patients suggest that immunomodulatory mechanisms may operate during infection. Determinations of CD19+ B-cell levels in the CSF could improve disease staging.

15009. **Buscher, P., Mumba Ngoyi, D., Kabore, J., Lejon, V., Robays, J., Jamonneau, V., Bebronne, N., Van der Veken, W. & Bieler, S., 2009.** Improved models of mini anion exchange centrifugation technique (mAECT) and modified single centrifugation (MSC) for sleeping sickness diagnosis and staging. *PLoS Neglected Tropical Diseases*, **3** (11): e471.

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

15010. **Hainard, A., Tiberti, N., Robin, X., Lejon, V., Ngoyi, D. M., Matovu, E., Enyaru, J. C., Fouda, C., Ndung'u, J. M., Lisacek, F., Muller, M., Turck, N. & Sanchez, J. C., 2009.** A combined CXCL10, CXCL8 and H-FABP panel for the staging of human African trypanosomosis patients. *PLoS Neglected Tropical Diseases*, **3** (6): e459.

Biomedical Proteomics Research Group, Medical University Centre, Geneva, Switzerland.

Human African trypanosomosis (HAT), also known as sleeping sickness, is a parasitic tropical disease. It progresses from the first, haemolymphatic stage to a neurological second stage due to invasion of parasites into the central nervous system (CNS). As treatment depends on the stage of disease, there is a critical need for tools that efficiently discriminate the two stages of HAT. We hypothesized that markers of brain damage discovered by proteomic strategies and inflammation-related proteins could individually or in combination indicate the CNS invasion by the parasite. Cerebrospinal fluid (CSF) originated from parasitologically confirmed *Trypanosoma brucei gambiense* patients. Patients were staged on the basis of CSF white blood cell (WBC) count and presence of parasites in CSF. One hundred samples were analysed: 21 from stage 1 (no trypanosomes in CSF and ≤ 5 WBC/ μ L) and 79 from stage 2 (trypanosomes in CSF and/or > 5 WBC/ μ L) patients. The concentration of H-FABP, GSTP-1 and S100beta in CSF was measured by ELISA. The levels of thirteen inflammation-related proteins (IL-1ra, IL-1beta, IL-6, IL-9, IL-10, G-CSF, VEGF, IFN-gamma, TNF-alpha, CCL2, CCL4, CXCL8 and CXCL10) were determined by bead suspension arrays. Results showed that CXCL10 most accurately distinguished stage 1 and stage 2 patients, with a sensitivity of 84 percent and specificity of 100 percent. Rule Induction Like (RIL) analysis defined a panel characterized by CXCL10, CXCL8 and H-FABP that improved the detection of stage 2 patients to 97 percent sensitivity and 100 percent specificity. This study highlights the value of CXCL10 as a single biomarker for staging *T. b. gambiense*-infected HAT patients. Further combination of CXCL10 with H-FABP and CXCL8 results in a panel that efficiently rules in stage 2 HAT patients. As these molecules could potentially be markers of other CNS infections and disorders, these results should be validated in a larger multi-centric cohort including other inflammatory diseases such as cerebral malaria and active tuberculosis.

15011. **Kager, P. A., Schipper, H. G., Stam, J. & Majoie, C. B., 2009.** Magnetic resonance imaging findings in human African trypanosomosis: a four-year follow-up study in a patient and review of the literature. *American Journal of Tropical Medicine and Hygiene*, **80** (6): 947-952.

Center for Infection and Immunity, Department of Infectious Diseases, Tropical Medicine and AIDS, Academic Medical Center, University of Amsterdam, Amsterdam, The Netherlands. [p.a.kager@amc.uva.nl].

Serial magnetic resonance imaging (MRI) was performed up to four years after treatment in a patient with *Trypanosoma brucei gambiense* infection. Four years after treatment and cure abnormalities were still present, although the patient led a normal social life, without physical and mental impairments. The literature on MRI in human African trypanosomosis is reviewed. The MRI is useful to discriminate between encephalitis induced by trypanosomosis and post-treatment reactive encephalopathy, a severe and often fatal complication of treatment, in particular of treatment with arsenicals. The MRI is not useful for diagnosis of human African trypanosomosis (HAT).

15012. **Kristensson, K., Nygard, M., Bertini, G. & Bentivoglio, M., 2009.** African trypanosome infections of the nervous system: parasite entry and effects on sleep and synaptic functions. *Progress in Neurobiology*. **e Publication ahead of print, December 6.**

Department of Neuroscience, Karolinska Institutet, Retzius vag 8, Stockholm SE-171 77, Sweden.

The extracellular parasite *Trypanosoma brucei* causes human African trypanosomosis (HAT), also known as sleeping sickness. Trypanosomes are transmitted by tsetse flies and HAT occurs in foci in sub-Saharan Africa. The disease, which is invariably lethal if untreated, evolves in a first haemo-lymphatic stage, progressing to a second meningo-encephalitic stage when the parasites cross the blood-brain barrier. At first, trypanosomes are restricted to circumventricular organs and choroid plexus in the brain outside the blood-brain barrier, and to dorsal root ganglia. Later, parasites cross the blood-brain barrier at post-capillary venules through a multi-step process similar to that of lymphocytes. Accumulation of parasites in the brain is regulated by cytokines and chemokines. Trypanosomes can alter neuronal function and the most prominent manifestation is represented by sleep alterations. These are characterized in HAT and experimental rodent infections by disruption of the sleep-wake 24h cycle and internal sleep structure. Trypanosome infections alter also some, but not all, other endogenous biological rhythms. A number of neural pathways and molecules may be involved in such effects. Trypanosomes secrete prostaglandins including the somnogenic PGD₂, and they interact with the host's immune system to cause release of pro-inflammatory cytokines. From the sites of early localization of parasites in the brain and meninges, such molecules could affect adjacent brain areas implicated in sleep-wakefulness regulation, including the suprachiasmatic nucleus and its downstream targets, to cause the changes characteristic of the disease. This raises challenging issues on the effects of cytokines on synaptic functions potentially involved in sleep-wakefulness alterations.

15013. **Nadjm, B., Van Tulleken, C., Macdonald, D. & Chiodini, P. L., 2009.** East African trypanosomiasis in a pregnant traveler. *Emerging Infectious Diseases*, **15** (11): 1866-1867.

The Hospital for Tropical Diseases, London, UK; London School of Hygiene and Tropical Medicine, London; UK; Chelsea and Westminster Hospital, London, UK. [behzad.nadjm@lshtm.ac.uk].

We describe a case of *T. brucei rhodesiense* infection occurring in the pregnant traveler. The 32-year-old woman, 20 weeks pregnant, returned from a nine-day safari trip to Tanzania 8 days before coming to a hospital in London. She described the short history of fever, headache, and soft-tissue swelling of the forehead with severe regional adenopathy. She had demonstrated necrosis of skin (chancre). Blood tests showed anaemia (hemoglobin 9.5 g / dL), leukopenia (1.8×10^9 cells / L) and thrombocytopenia (60×10^9 cells / L). The film showed blood trypomastigotes of *T. brucei rhodesiense*. Suramin treatment was unavailable, but because of her deteriorating clinical state, she was treated with a dose of pentamidine (4 mg / kg) before suramin was obtained. Suramin was begun 36 hours after admission, initially at 5 mg / kg and increased over the next two doses up to 1 g. During the next 48 hours, her fever resolved, and serial blood films showed clearance of the parasites from the blood. The cerebrospinal fluid in the sample showed signs of stage II disease, and the patient continued on suramin, completing the course as an outpatient. Her pregnancy was closely monitored, and she gave birth at term to a healthy baby girl.

15014. **Pays, E. & Vanhollebeke, B., 2009.** Human innate immunity against African trypanosomes. *Current Opinion in Immunology*, **21** (5): 493-498.

Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, 12, rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium.

Humans are naturally resistant to infection by the African trypanosome prototype *Trypanosoma brucei brucei*, and only two variant clones of this parasite can avoid this innate immunity and cause sleeping sickness. The resistance to *T. brucei* is due to serum complexes associating apolipoprotein A-1 (apoA1) with two primate-specific proteins, apolipoprotein L-1 (apoL1) and haptoglobin-related protein (Hpr). We discuss recent advances on the respective functions of apoL1 and Hpr in this system. ApoL1 was found to share structural and functional similarities with proteins of the apoptotic Bcl2 family, and to kill trypanosomes through anionic pore formation in the lysosomal membrane of the parasite. In association with haemoglobin (Hb), Hpr was found to promote the binding of the trypanolytic complexes to a haptoglobin (Hp)-Hb receptor of the trypanosome surface, hereby facilitating the internalization of apoL1. Hpr or apoL1 deficiency respectively leads to the reduction or abolishment of human protection against *T. brucei*.

(c) TREATMENT

[See also **32**: 14970, 14971, 15074].

15015. **Bacchi, C. J., 2009.** Chemotherapy of human African trypanosomiasis. *Interdisciplinary Perspectives on Infectious Diseases*, **2009**: 195040.

Department of Biological and Health Sciences, Haskins Laboratory, Pace University, New York, NY 10038, USA.

Human African trypanosomosis is a centuries-old disease which has disrupted sub-Saharan Africa in both physical suffering and economic loss. This article presents an update of the classic chemotherapeutic agents in use for >50 years and the recent development of promising non-toxic combination chemotherapy suitable for use in rural clinics.

15016. **Kazibwe, A. J., Nerima, B., de Koning, H. P., Maser, P., Barrett, M. P. & Matovu, E., 2009.** Genotypic status of the TbAT1/P2 adenosine transporter of *Trypanosoma brucei gambiense* isolates from Northwestern Uganda following melarsoprol withdrawal. *PLoS Neglected Tropical Diseases*, **3** (9): e523.

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

The development of arsenical and diamidine resistance in *Trypanosoma brucei* is associated with loss of drug uptake by the P2 purine transporter as a result of alterations in the corresponding *T. brucei* adenosine transporter 1 gene (TbAT1). Previously, specific TbAT1 mutant type alleles linked to melarsoprol treatment failure were significantly more prevalent in *T. b. gambiense* from relapse patients at Omugo health centre in Arua district. Relapse rates of up to 30 percent prompted a shift from melarsoprol to eflornithine (alpha-difluoromethylornithine, DFMO) as first-line treatment at this centre. The aim of this study was to determine the status of TbAT1 in recent isolates collected from *T. b. gambiense* sleeping sickness patients from Arua and Moyo districts in Northwestern Uganda after this shift in first-line drug choice. Blood and cerebrospinal fluids of consenting patients were collected for DNA preparation and subsequent amplification. All 105 isolates from Omugo that we successfully analysed by PCR-RFLP possessed the TbAT1 wild type allele. In addition, PCR/RFLP analysis was performed for 74 samples from Moyo, where melarsoprol is still the first line drug; 61 samples displayed the wild genotype while six were mutant and seven had a mixed pattern of both mutant and wild-type TbAT1. The melarsoprol treatment failure rate at Moyo over the same period was nine out of 101 stage II cases that were followed up at least once. Five of the relapse cases harboured mutant TbAT1, one had the wild type, while no amplification was achieved from the remaining three samples. The apparent disappearance of mutant alleles at Omugo may correlate with melarsoprol withdrawal as first-line treatment. Our results suggest that melarsoprol could successfully be reintroduced following a time lag subsequent to its replacement. A field-applicable test to predict melarsoprol treatment outcome and identify patients for whom the drug can still be beneficial is clearly required. This will facilitate cost-effective management of HAT in rural resource-poor settings, given that eflornithine has a much higher logistical requirement for its application.

15017. **Marin-Neto, J. A., Rassi, A., Jr., Avezum, A., Jr., Mattos, A. C., Rassi, A., Morillo, C. A., Sosa-Estani, S. & Yusuf, S., 2009.** The BENEFIT trial: testing the hypothesis that trypanocidal therapy is beneficial for patients with chronic Chagas heart disease. *Memórias do Instituto Oswaldo Cruz*, **104 Suppl 1**: 319-324.

Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brazil. [marin_net@yahoo.com].

Among the pathophysiological derangements operating in the chronic phase of Chagas disease, parasite persistence is likely to constitute the main mechanism of myocardial injury in patients with chronic chagasic cardiomyopathy. The presence of *Trypanosoma cruzi* in the heart causes a low-grade, but relentless, inflammatory process and induces myocardial autoimmune injury. These facts suggest that trypanocidal therapy may positively impact the clinical course of patients with chronic Chagas heart disease. However, the experimental and clinical evidence currently available is insufficient to support the routine use of aetiologic treatment in these patients. The BENEFIT project--Benznidazole Evaluation for Interrupting Trypanosomiasis--is an international, multicentre, double-blind, placebo-controlled trial of trypanocidal treatment with benznidazole in patients with chronic Chagas heart disease. This project is actually comprised of two studies. The pilot study investigates whether aetiologic treatment significantly reduces parasite burden, as assessed by polymerase chain reaction-based techniques and also determines the safety and tolerability profile of the trypanocidal drug in this type of chagasic population. The full-scale study determines whether anti-trypanosomal therapy with benznidazole reduces mortality and other major cardiovascular clinical outcomes in patients with chronic Chagas heart disease.

15018. **McKerrow, J. H., Doyle, P. S., Engel, J. C., Podust, L. M., Robertson, S. A., Ferreira, R., Saxton, T., Arkin, M., Kerr, I. D., Brinen, L. S. & Craik, C. S., 2009.** Two approaches to discovering and developing new drugs for Chagas disease. *Memórias do Instituto Oswaldo Cruz*, **104 Suppl 1**: 263-269.

Sandler Center at Mission Bay, University of California, San Francisco, CA 94158-2330, USA. [jmck@cgl.ucsf.edu].

This review focuses on two general approaches carried out at the Sandler Center, University of California, San Francisco, to address the challenge of developing new drugs for the treatment of Chagas disease. The first approach is target-based drug discovery, and two specific targets, cytochrome P450 CYP51 and cruzain (aka cruzipain), are discussed. A “proof of concept” molecule, the vinyl sulfone inhibitor K777, is now a clinical candidate. The preclinical assessment compliance for filing as an Investigational New Drug with the United States Food and Drug Administration (FDA) is presented, and an outline of potential clinical trials is given. The second approach to identifying new drug leads is parasite phenotypic screens in culture. The development of an assay allowing high throughput screening of *Trypanosoma cruzi* amastigotes in skeletal muscle cells is presented. This screen has the advantage of not requiring specific strains of parasites, so it could be used with field isolates, drug resistant strains or laboratory strains. It is optimized for robotic liquid handling and has been validated through a screen of a library of FDA-approved drugs identifying 65 hits.

15019. **Opigo, J. & Woodrow, C., 2009.** NECT trial: more than a small victory over sleeping sickness. *Lancet*, **374** (9683): 7-9.

District Health Services, Moyo District Local Government, Moyo, Uganda.

The natural history of central nervous system infection by *Trypanosoma brucei gambiense* consists of a distinctive neurological syndrome (sleeping sickness) proceeding to inevitable death. For more than 50 years intravenous melarsoprol has been the most common

therapeutic approach, but this arsenical compound can cause a reactive encephalopathy with high risk of mortality and shows falling efficacy in certain areas. Eflornithine is an efficacious alternative with fewer side-effects, but the need for its 6-hourly administration via slow infusion, over 14 d, has limited uptake in resource-poor settings. Oral nifurtimox shows too low an efficacy for routine use as monotherapy but has been tested recently in combination with eflornithine, yielding encouraging data on efficacy and side-effect profile. In *The Lancet* today, Gerardo Priotto and colleagues (see below) present an open-label randomised trial comparing standard eflornithine (400 mg/kg per day in 6-hourly infusions for 14 days) with nifurtimox-eflornithine combination therapy (NECT: oral nifurtimox 15 mg/kg/d for 10 d, eflornithine 400 mg/kg/d in 12-hourly infusions for 7 d) in adults with stage II African trypanosomosis. Both the trial methodology and results are noteworthy. The study design was non-inferiority, a pragmatic decision in view of the predicted cure rate of more than 90 percent. Non-inferiority trials demand robust diagnosis, treatment, and follow-up (notoriously difficult in this context), because weak methodology tends to dilute differences in efficacy which increases the chance of a type I error (false conclusion of non-inferiority). In this regard, the study performed optimally with a completed follow-up rate of 93 percent, a truly remarkable figure in view of the logistical challenge of doing lumbar punctures over an 18-month period in nearly 300 patients living in remote communities. As it turned out, in the planned primary outcome analyses of efficacy, NECT seemed superior to eflornithine alone (cure rates of around 97 percent vs 92 percent). Even the worst-case sensitivity analysis, in which losses to follow-up were regarded as failures, confirmed non-inferiority. Adverse events were generally fewer in the NECT group. These findings suggest that NECT has typical advantages of a combination therapy: equivalent or improved efficacy and reduced side-effects. Furthermore the reduced frequency, duration of course, and total quantity of eflornithine infusion in NECT favour its use in resource-poor settings, in view of the savings in transport and equipment costs as well as staff time. On theoretical grounds, the combination should inhibit the development of resistance to the individual component drugs, as seen for various other infections. WHO has already endorsed the study's findings by entering NECT into its Essential Medicines List. It should be pointed out that there has not been a direct comparison of NECT with melarsoprol, or nifurtimox–melarsoprol, a combination favoured by some practitioners that proved efficacious in another large trial. However, we believe that there is now a strong evidence base to support promotion of NECT within national treatment strategies. Despite the optimism generated by today's trial, innumerable challenges remain, including the urgent need to develop improved treatments for the earlier haemolymphatic phase (stage I) of African trypanosomosis. Despite best efforts, there are still no reports of successfully completed phase III randomised trials in stage I, in which treatment (pentamidine administered by 7–10 daily intramuscular injections) has also remained unchanged for half a century. In our experience it is paradoxically more difficult to recruit and follow up patients with milder clinical manifestations than those in stage II disease, and these studies will require particularly intense support from sponsors and collaboration from all partners. There is also room to refine diagnosis as well as develop coherent strategies for control and surveillance. Today's study with NECT shows the way forward, setting a high bar in terms of trial methodology that other studies should aim to replicate. The success of this study depended on collaboration between a wide variety of agencies, including the Drugs for Neglected Disease Initiative and Médecins Sans Frontières (the trial's sponsors), several academic centres, and the national trypanosomosis programmes of the Popular Republic of the Congo and the Democratic Republic of the Congo.

Fundamentally, this project builds on the efforts of countless individuals in sleeping sickness teams across Africa who work in indescribably difficult conditions year after year with a positive and indomitable spirit. This potent combination has produced a study that in every respect rivals those in diseases for which research receives vastly superior funds.

15020. Priotto, G., Kasparian, S., Mutombo, W., Ngouama, D., Ghorashian, S., Arnold, U., Ghabri, S., Baudin, E., Buard, V., Kazadi-Kyanza, S., Ilunga, M., Mutangala, W., Pohlig, G., Schmid, C., Karunakara, U., Torreele, E. & Kande, V., 2009. Nifurtimox-eflornithine combination therapy for second-stage African *Trypanosoma brucei gambiense* trypanosomiasis: a multicentre, randomized, phase III, non-inferiority trial. *Lancet*, **374** (9683): 56-64.

Epicentre, Paris, France. [gpriotto@epicentre.msf.org].

Human African trypanosomosis (HAT; sleeping sickness) caused by *Trypanosoma brucei gambiense* is a fatal disease. Current treatment options for patients with second-stage disease are toxic, ineffective, or impractical. We assessed the efficacy and safety of nifurtimox-eflornithine combination therapy (NECT) for second-stage disease compared with the standard eflornithine regimen through a multicentre, randomized, open-label, active control, phase III, non-inferiority trial done at four HAT treatment centres in the Republic of the Congo and the Democratic Republic of the Congo. Patients aged 15 years or older with confirmed second-stage *T. b. gambiense* infection were randomly assigned by computer-generated randomization sequence to receive intravenous eflornithine (400 mg/kg per day, every 6 h; n=144) for 14 days or intravenous eflornithine (400 mg/kg per day, every 12 h) for seven days with oral nifurtimox (15 mg/kg per day, every 8 h) for ten days (NECT; n=143). The primary endpoint was cure (defined as absence of trypanosomes in body fluids and a leucocyte count ≤ 20 cells per μL) 18 months after treatment. Efficacy analyses were done in the intention-to-treat (ITT), modified ITT, and per-protocol (PP) populations. The non-inferiority margin for the difference in cure rates was defined as 10 percent. This study is registered with ClinicalTrials.gov, number NCT00146627. One patient from the eflornithine group absconded after receiving the first dose without any type of assessment done, and was excluded from all analyses. In the ITT population, 131 (91.6 percent) of 143 patients assigned to eflornithine and 138 (96.5 percent) of 143 patients assigned to NECT were cured at 18 months (difference -4.9 percent, one-sided 95 percent CI -0.3; $p < 0.0001$). In the PP population, 122 (91.7 percent) of 133 patients in the eflornithine group and 129 (97.7 percent) of 132 in the NECT group were cured at 18 months (difference -6.0 percent, one-sided 95 percent CI -1.5; $P < 0.0001$). Drug-related adverse events were frequent in both groups; 41 (28.7 percent) patients in the eflornithine group and 20 (14.0 percent) in the NECT group had major (grade 3 or 4) reactions, which resulted in temporary treatment interruption in nine and one patients, respectively. The most common major adverse events were fever (n=18), seizures (n=6), and infections (n=5) in the eflornithine group, and fever (n=7), seizures (n=6), and confusion (n=2) in the NECT group. There were four deaths, which were regarded as related to the study drug (eflornithine, n=3; NECT, n=1). It is concluded that the efficacy of NECT is non-inferior to that of eflornithine monotherapy. Since this combination treatment also presents safety advantages, is easier to administer (i.e. infusion every 12 h for seven days vs every 6 h for 14 days), and potentially protective against the emergence of resistant parasites, it is suitable for first-line use in HAT control programmes.

15021. **Van den Enden, E., Vlieghe, E., Demeester, R., Ieven, G., Jansens, H. & Van den Hauwe, L., 2009.** A traveler with neurobrucellosis. *Travel Medicine and Infectious Disease*, **7** (4): 215-218.

Institute of Tropical Medicine, Department of Clinical Sciences, Nationalestraat 155, 2000 Antwerp, Belgium. [evdenden@itg.be].

An Indian traveler developed fever and neurological symptoms after a visit to East Africa. He was treated with suramin, melarsoprol and prednisolone for presumed East African trypanosomosis. His condition deteriorated and cerebral lesions developed. Neurobrucellosis was diagnosed. Combination antibiotic therapy led to gradual clinical improvement and regression of the brain lesions. Misdiagnosis of East African trypanosomosis followed by treatment with potentially lethal medication should be avoided by not relying on insufficient evidence during the diagnostic process.

15022. **Vodnala, S. K., Ferella, M., Lunden-Miguel, H., Betha, E., van Reet, N., Amin, D. N., Oberg, B., Andersson, B., Kristensson, K., Wigzell, H. & Rottenberg, M. E., 2009.** Preclinical assessment of the treatment of second-stage African trypanosomiasis with cordycepin and deoxycoformycin. *PLoS Neglected Tropical Diseases*, **3** (8): e495.

Department of Microbiology, Tumor and Cell Biology, Karolinska Institute, Stockholm, Sweden.

There is an urgent need to substitute the highly toxic compounds still in use for treatment of the encephalitic stage of human African trypanosomosis (HAT). We here assessed the treatment with the doublet cordycepin and the deaminase inhibitor deoxycoformycin for this stage of infection with *Trypanosoma brucei*. Cordycepin was selected as the most efficient drug from a direct parasite viability screening of a compound library of nucleoside analogues. The minimal number of doses and concentrations of the drugs effective for treatment of *T. b. brucei* infections in mice were determined. Oral, intraperitoneal or subcutaneous administrations of the compounds were successful for treatment. The doublet was effective for treatment of late stage experimental infections with human pathogenic *T. b. rhodesiense* and *T. b. gambiense* isolates. Late stage infection treatment diminished the levels of inflammatory cytokines in the brains of infected mice. Incubation with cordycepin resulted in programmed cell death followed by secondary necrosis of the parasites. *T. b. brucei* strains developed resistance to cordycepin after culture with increasing concentrations of the compound. However, cordycepin-resistant parasites showed diminished virulence and were not cross-resistant to other drugs used for treatment of HAT, i.e. pentamidine, suramin and melarsoprol. Although resistant parasites were mutated in the gene coding for P2 nucleoside adenosine transporter, P2 knockout trypanosomes showed no altered resistance to cordycepin, indicating that absence of the P2 transporter is not sufficient to render the trypanosomes resistant to the drug. Altogether, our data strongly support testing of treatment with a combination of cordycepin and deoxycoformycin as an alternative for treatment of second-stage and/or melarsoprol-resistant HAT.

15023. **Wenzler, T., Boykin, D. W., Ismail, M. A., Hall, J. E., Tidwell, R. R. & Brun, R., 2009.** New treatment option for second-stage African sleeping sickness: *in vitro* and *in vivo* efficacy of aza analogues of DB289. *Antimicrobial Agents and Chemotherapy*, **53** (10): 4185-4192.

Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, Basel, Switzerland. [tanja.wenzler@unibas.ch].

African sleeping sickness is a fatal parasitic disease, and all drugs currently in use for treatment have strong liabilities. It is essential to find new, effective, and less toxic drugs, ideally with oral application, to control the disease. In this study, the aromatic diamidine DB75 (furamidine) and two aza analogs, DB820 and DB829 (CPD-0801), as well as their methoxyamidine prodrugs and amidoxime metabolites, were evaluated against African trypanosomes. The active parent diamidines showed similar *in vitro* profiles against different *Trypanosoma brucei* strains, melarsoprol- and pentamidine-resistant lines, and a P2 transporter knockout strain (AT1KO), with DB75 as the most trypanocidal molecule. In the *T. b. rhodesiense* strain STIB900 acute mouse model, the aza analogues DB820 and DB829 demonstrated activities superior to that of DB75. The aza prodrugs DB844 and DB868, as well as two metabolites of DB844, were orally more potent in the *T. b. brucei* strain GVR35 mouse central nervous system (CNS) model than DB289 (pafuramidine maleate). Unexpectedly, the parent diamidine DB829 showed high activity in the mouse CNS model by the intraperitoneal route. In conclusion, DB868 with oral and DB829 with parenteral application are potential candidates for further development of a second-stage African sleeping sickness drug.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

15024. **Dayo, G. K., Bengaly, Z., Messad, S., Bucheton, B., Sidibe, I., Cene, B., Cuny, G. & Thevenon, S., 2009.** Prevalence and incidence of bovine trypanosomosis in an agro-pastoral area of southwestern Burkina Faso. *Research in Veterinary Science*. **In press, corrected proof.**

IRD, UMR Trypanosomes, TA A-17/A Campus International de Baillarguet, 34398 Montpellier Cedex 5, France; CIRDES, BP 454 Bobo Dioulasso, Burkina Faso.

The present study was conducted to determine the prevalence and incidence of trypanosomosis and to investigate some factors influencing them in an agro-pastoral area of southwestern Burkina Faso. A total of 363 crossbred cattle (Baoule-zebu peul), which were bred under natural trypanosomosis challenge, were monitored monthly for parasitaemia, packed cell volume (PCV) and serological analyses over two years. The parasitological prevalence estimated at the beginning of the survey using the buffy coat technique (BCT) was 7.54 percent. As much as 66.7 percent of all trypanosome infections were due to *Trypanosoma vivax*, 23.8 percent due to *Trypanosoma congolense* and 9.5 percent due to *T. vivax/T. congolense* mixed infections. The monthly serological incidence varied from 0.29

percent to 19.29 percent. The season was the most important factor influencing the serological prevalence and incidence and the animal PCV. The dry hot season is associated with increasing seroprevalences and incidences and consequently a decreasing average of PCV. In addition, an important spatial heterogeneity was observed.

15025. **Desquesnes, M., Kamyinkird, K., Pruvot, M., Kengradomkij, C., Bossard, G., Sarataphan, N. & Jittapalpong, S., 2009.** Antibody-ELISA for *Trypanosoma evansi*: application in a serological survey of dairy cattle, Thailand, and validation of a locally produced antigen. *Preventive Veterinary Medicine*, **90** (3-4): 233-241.

Center for International Cooperation in Agronomical Research for Development (CIRAD), Department of Biological Systems (Bios), UMR177-Trypanosomes (IRD-CIRAD), Montpellier F-34000, France. [marc.desquesnes@cirad.fr].

Trypanosoma evansi is generally considered a mild pathogen in bovines. However, in Asia, acute and chronic signs have been observed in cattle, with high levels of parasitaemia, abortion and death. Investigations in Asian cattle are needed to better understand this epidemiological situation. To generate comparable data at a regional level, development and standardization of an antibody-enzyme linked immunosorbent assay for *T. evansi* was initiated and applied in an epidemiological survey carried out in dairy cattle in Thailand. A batch of 1 979 samples was collected from dairy farms located throughout the country's four regions. Soluble *T. evansi* antigens initially produced in France were also produced in Thailand for comparison and technology transfer. Screening of 500 samples allowed us to identify reference samples and to determine the cut-off value of the ELISA. Seropositive animals - some of them confirmed by PCR - were found in the four regions, in 12 out of 13 provinces, in 22 out of 31 districts, in 56 farms out of 222 (25 percent, 95 percent CI+/-6 percent) and in 163 animals out of 1979 (8.2 percent, 95 percent CI+/-1.2 percent). Estimated seroprevalence in 35 farms ranged between 1 percent and 30 percent, and in 21 farms it was >30 percent. Approximately 25 percent of survey cattle were exposed to the infection, in various situations. A sub-sample of 160 sera was tested using both antigens. Wilcoxon's ($Z=1.24$; $P=0.22$) and McNemars's tests ($\text{CHI}^2=3.55$; $p=0.09$) did not show any significant differences, showing that the locally produced antigen is suitable for further evaluation in the surrounding countries. Use of this standardized serological method will broaden knowledge of the prevalence and impact of the disease at the regional level in South-East Asia. Further validation of this ELISA will be necessary in other host species such as buffalo, horse and pig.

15026. **Jing, Z., Magona, J. W., Sakurai, T., Thekiso, O. M., Otim, C. P., Sugimoto, C. & Inoue, N., 2009.** A field study to estimate the prevalence of bovine African trypanosomosis in Butaleja District, Uganda. *Journal of Veterinary Medical Science*, **71** (4): 525-527.

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

The prevalence of bovine trypanosomosis was determined from a total of 203 blood samples collected from Butaleja District, eastern Uganda. All samples were examined by the microhaematocrit centrifuge test (MHC), PCR and ELISA. ELISA was performed in

accordance with the OIE standard procedures using *Trypanosoma brucei gambiense* procyclic form crude antigens. PCR were utilized to identify the species and the subspecies of trypanosome. The overall prevalence of bovine African trypanosomosis was 8.9 percent by MHC, and 45.3 percent by the ELISA. Since a substantial number (12 out of 18) of MHC positive samples were negative in the PCR tests, we could not determine the most epidemic trypanosome species in the studied area. Nevertheless, the PCR results suggest that the most prevalent trypanosome was *T. b. brucei* (31/203), followed by *T. congolense* (6/203). In addition, only a few (3/203) mixed infections of *T. b. brucei* and *T. congolense* were detected by the PCR. Results obtained from this study indicate that bovine trypanosomosis is endemic in Butaleja District, Uganda.

15027. **Keck, N., Herder, S., Kaba, D., Solano, P., Gomez, J., Cuny, G. & Davoust, B., 2009.** Epidemiological study of canine trypanosomosis in an urban area of Ivory Coast. *Parasite*, **16** (4): 305-308.

Laboratoire de recherches et de coordination sur les trypanosomoses, Institut de recherche pour le développement / Centre de coopération internationale en recherche agronomique pour le développement, TA A-17/G 34398 Montpellier Cedex 5, France.

Following the confirmation of cases of trypanosomosis in military working dogs, a cross-sectional study was undertaken to evaluate the source of infection and determine the prevalence of canine infection with *Trypanosoma congolense* in the urban focus of Abidjan, Ivory Coast. Blood from 123 dogs was collected and subjected to PCR using specific primers for *Trypanosoma congolense* "forest type". In addition, an entomological study was conducted in an urban area near the forest surrounding the military camp. The observed prevalence was 30.1 percent and PCR positivity to *Trypanosoma congolense* was not significantly associated with sex or age of animals. This study demonstrates the high contamination rate of dogs in enzootic zones, the potential risk of introduction of the disease in free animal populations and the ability of *Glossina palpalis* to adapt to urban areas and to transmit trypanosomosis in such areas. The factors leading to a possible emergence of canine trypanosomosis in enzootic zones need further investigation.

15028. **Konnai, S., Mekata, H., Mingala, C. N., Abes, N. S., Gutierrez, C. A., Herrera, J. R., Dargantes, A. P., Witola, W. H., Cruz, L. C., Inoue, N., Onuma, M. & Ohashi, K., 2009.** Development and application of a quantitative real-time PCR for the diagnosis of Surra in water buffaloes. *Infection, Genetics and Evolution*, **9** (4): 449-452.

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

Trypanosoma evansi causes the disease called Surra in domestic animals, which is of great economic importance in South Asian countries. In order to improve the diagnosis of Surra, we endeavored to develop a real-time PCR assay for the detection and quantification of parasites in water buffaloes using specific primers for the *T. evansi* Rode Trypanozoon antigen type (RoTat) 1.2 Variable Surface Glycoprotein (VSG) gene, which is a known diverse DNA region in trypanosomes. The quantitative detection limit of the assay was 10²

trypanosomes per mL of blood, and the identity of the amplicon was confirmed in all assays by melting curve analysis. To evaluate the clinical applicability of this procedure, detection and estimation of parasitaemia in blood samples obtained from water buffaloes and horses were conducted. *T. evansi* was detected in 17/607 (2.8 percent) blood samples, with parasitaemia levels ranging from >10 to 10^7 parasites per mL of blood. Interestingly, out of the 17 PCR positive animals, three had previously received trypanocidal treatment and one had a history of abortion. These data indicate that real-time PCR for the estimation of putative parasitaemia levels is a quantitatively and objectively applicable technique for clinical diagnosis of Surra, and could help to understand the disease stage and risk of transmission of *T. evansi*.

15029. **Mekata, H., Konnai, S., Witola, W. H., Inoue, N., Onuma, M. & Ohashi, K., 2009.** Molecular detection of trypanosomes in cattle in South America and genetic diversity of *Trypanosoma evansi* based on expression-site-associated gene 6. *Infection, Genetics and Evolution*, **9** (6): 1301-1305.

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

In South American countries, trypanosomosis as a result of *Trypanosoma evansi* and *Trypanosoma vivax* infections causes significant economic losses in livestock. The objectives of this study were to characterize the epidemiology of bovine trypanosomosis in South America and to draw a comparison between South American and Asian *T. evansi* isolates based on the polymorphisms in their transferrin receptor encoding gene 6. We assessed the prevalence rates of *T. evansi* and *T. vivax* infections in cattle in different regions of Peru and Bolivia using the polymerase chain reaction (PCR) and found that, in Lima and Pucallpa in the Republic of Peru, *T. evansi* infection rates were 5.8 percent (6/104) and 2.5 percent (5/195), respectively, while in Santa Cruz, Republic of Bolivia, the infection rate for *T. evansi* was 11.5 percent (59/510). The prevalence rates of *T. vivax* in Lima and Santa Cruz were 3.8 percent (4/104) and 0.9 percent (5/510), respectively. In *T. evansi*, uptake of host transferrin is mediated by a receptor derived from the two expression site-associated genes 6 and 7 (ESAG6 and ESAG7). We previously showed that the ESAG6 depicts genetic diversity among different isolates of *T. evansi* in Asia. In this study, we cloned and sequenced the ESAG6 genes from *T. evansi* isolates from South America, and found, in addition to some of the previously observed variants, 20 novel variants of ESAG6 genes which could be categorized into three new clades among the various isolates. To conclude, the results obtained in this study suggest that *T. evansi* isolates from South America are more diverse than the Asian isolates.

15030. **Oliveira, J. B., Hernandez-Gamboa, J., Jimenez-Alfaro, C., Zeledon, R., Blandon, M. & Urbina, A., 2009.** First report of *Trypanosoma vivax* infection in dairy cattle from Costa Rica. *Veterinary Parasitology*, **163** (1-2): 136-139.

Escuela de Medicina Veterinaria, Universidad Nacional, APDO 86-3000, Barreal de Heredia, Heredia, Costa Rica. [jaquelo@medvet.una.ac.cr].

An outbreak of haemoparasitoses occurred from October 2007 to July 2008 in cattle from the district of Rio Cuarto, province of Alajuela, Costa Rica. Fifty animals of various

ages out of 450 Brown Swiss were affected. The animals presented fever, severe anemia, jaundice, abortion or premature birth, loss of appetite, decrease milk production and accentuated weight loss in a short period of time. Haemoparasites were observed in the blood smears: *Anaplasma marginale* was present in 17 animals (60.7 percent); *Trypanosoma vivax* in nine (32.1 percent) and *Babesia bovis* in two (7.1 percent). Three of the animals (10.7 percent) had a mixed infection with *T. vivax* and *A. marginale*. After treatment, all the animals were clinically recovered and subsequent blood samplings showed no parasites. Data suggest that the outbreak might be related to a decrease in the availability and quality of the pastures due to very heavy rainfalls during the year 2007, as well as an increase in the abundance of *Boophilus microplus* and *Stomoxys calcitrans*. This is the first report of the presence of *T. vivax* in Costa Rica.

15031. **Savage, A. F., Robert, V., Goodman, S. M., Raharimanga, V., Raherilalao, M. J., Andrianarimisa, A., Arie, F. & Greiner, E. C., 2009.** Blood parasites in birds from Madagascar. *Journal of Wildlife Diseases*, **45** (4): 907-920.

Department of Infectious Diseases and Pathology, College of Veterinary Medicine, University of Florida, Box 110880, Gainesville, Florida 32611, USA. [haemosporida@yahoo.com].

Madagascar has long been recognized for its unique and diverse biota. In particular, significant effort has been made to establish baseline population data to better conserve the endemic avifauna. During field expeditions between 1993 and 2004, birds were mist-netted at 11 different sites, at elevations from 60 m to 2 050 m above sea level. Data on endemic status, forest type, and habitat preference were recorded. Thin blood films from 947 birds, belonging to 26 families and 64 species, were examined by light microscopy to determine the prevalence of blood parasites. Of these 947 birds, 30.7 percent were infected by at least one species of blood parasite, 26.8 percent of which were infected by more than one species. Species of *Haemoproteus* were the most prevalent (17.4 percent), followed by microfilariae (11.0 percent), *Leucocytozoon* spp. (9.4 percent), *Plasmodium* spp. (1.9 percent), *Trypanosoma* spp. (0.9 percent), and *Babesia* spp. (0.2 percent). Species level identifications confirmed the presence of 47 species of hemosporidians and trypanosomes, which is notably high and mirrors the diversity of their avian hosts. Eleven (23.4 percent) of these parasite species were new to science and thought to be endemic to the island. Significant differences in prevalence were observed by sample site, forest type (humid vs. dry), and habitat preference. Birds from all elevational zones sampled were infected, although not all parasite genera were present in each zone. Four of the six endemic avian families or subfamilies (*Bernieridae*, *Brachypteraciidae*, *Philepittinae* [*Eurylaimidae*], and *Vangidae*) were sampled and found to be parasitized. Of the families with the largest sample sizes, the *Zosteropidae* and *Ploceidae* had the highest prevalence of infection (65.6 percent and 49.3 percent, respectively). The vectors of haematozoan parasites in Madagascar are currently unknown. These results add to the current knowledge of avian parasitism in Madagascar and are of particular interest for the conservation of endemic species, as well as threatened or endangered populations.

15032. **Soltysiak, Z., Gorczykowski, M., Pawlas-Opiela, M., Chelmonska-Soyta, A. & Nowacki, W., 2009.** Accidental discovery of *Trypanosoma theileri* in the *in vitro*

culture of the heifer lymphocytes. *Polish Journal of Veterinary Science*, **12** (3): 395-398.

Parasitology Unit, Department of Internal and Parasitic Diseases with Clinic of Horses, Dogs and Cats Diseases, Wrocław University of Environmental and Life Sciences, C.K. Norwida 31, 50-375 Wrocław, Poland [zenon.soltysiak@up.wroc.pl].

The diagnostics of the *Trypanosoma* sp. invasion by means of the classic methods i.e. the methods of thin smears or thick drop or even the microhaematocrite method, especially when intensity of infection is low, is very difficult. In our climatic zone, trypanosomosis is usually considered as an exotic disease. An opportunistic model of the infection with the parasite and a lack of current data on the prevalence of *T. theileri* in the cattle in Poland mean that it is neglected as a potential reason for contamination of tissue cultures in cattle. We showed the presence of *T. theileri* in the culture of isolated lymphocytes from one of six heifers examined. It seems that the prevalence of the invasion of the parasite is not very intense but it should be considered as a possible threat for bovine cell culture. It is also worth including this parasitosis in the differential diagnostics of other diseases that are infectious and/or have symptoms of immunosuppression.

15033. **Sumbu, J., De Deken, R., Deckers, N., Mpiana, S., Kabambi, P., Tshilenge, G. & Boelaert, M., 2009.** Spatial variation of risk for pigs to contract trypanosomosis in farms situated in the peri-urban area of Kinshasa. *Parasite*, **16** (2): 153-159.

Laboratoire Vétérinaire de Kinshasa, Kinshasa, République Démocratique du Congo.

In an effort to understand better the transmission risk as well for the animal African trypanosomosis (AAT) as for the human trypanosomosis (HAT) in the peri-urban zone of Kinshasa, a serologic study was carried out in local pig farms from 2003 to 2005. An indirect ELISA was used to detect the presence of trypanosome antibodies in 1 240 pigs originating from 404 farms. Seropositivity was recorded in 155 farms (38 percent), but varied considerably according to the district. In 6 percent of the farms AAT could be confirmed by parasitological examination. Trapping sites (n = 367) established in the neighbourhood of pig farms made it possible to capture 1 935 tsetse flies (*Glossina fuscipes quanzensis*). Among 562 dissected flies 23 were found to harbour trypanosomes resulting in an infection rate of 4.1 percent. In the majority of the districts the transmission risk for animal trypanosomosis anticipated from the apparent vector densities was corroborated by the serology. Zones with strong indications of local AAT transmission were identified in several quarters of three peri-urban districts of Kinshasa: Mount-Ngafula, Ngaliema and N'Sele. An intensification of tsetse control activities in those sites of increased transmission risk is essential.

15034. **Thekiso, O. M., Bazie, R. S., Coronel-Servian, A. M., Sugimoto, C., Kawazu, S. & Inoue, N., 2009.** Stability of loop-mediated isothermal amplification (LAMP) reagents and its amplification efficiency on crude trypanosome DNA templates. *Journal of Veterinary Medical Science*, **71** (4): 471-475.

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

This study evaluated the stability of LAMP reagents when stored at 25 °C and 37 °C, and also assessed its detection efficiency on different DNA template preparations through amplifying DNA of *in vitro* cultured *T. b. brucei* from day 1 to day 15 of reagent storage. There were no significant differences ($P>0.05$) in detection sensitivity of LAMP among the reagents stored at these temperatures and -20 °C (the recommended storage temperature). LAMP using the reagents stored at the above-mentioned temperatures amplified serially diluted DNAs (genomic DNA extracted by phenol-chloroform method, FTA card and haemolysed blood) of *T. b. gambiense* (IL2343) with high sensitivity. Reactions were conducted on the reagents stored from 1 day to 30 days. LAMP detection sensitivity was poor when fresh blood was added as the DNA template directly into reactive solution. Results of this study demonstrated that LAMP has the potential to be used in field conditions for diagnosing trypanosome infections without being affected by the ambient temperatures of tropical and sub-tropical countries where trypanosomosis is endemic.

(b) PATHOLOGY AND IMMUNOLOGY

15035. **Batista, J. S., Oliveira, A. F., Rodrigues, C. M., Damasceno, C. A., Oliveira, I. R., Alves, H. M., Paiva, E. S., Brito, P. D., Medeiros, J. M., Rodrigues, A. C. & Teixeira, M. M., 2009.** Infection by *Trypanosoma vivax* in goats and sheep in the Brazilian semi-arid region: from acute disease outbreak to chronic cryptic infection. *Veterinary Parasitology*, **165** (1-2): 131-135.

Department of Animal Sciences, Federal Rural University of the Semi-Arid, Avenida Francisco Mota, Mossoro, RN, Brazil. [jaelsoares@hotmail.com].

A study was undertaken to investigate the role of *Trypanosoma vivax* in sheep and goat mortality and abortions in the Brazilian semi-arid region, where outbreaks had been previously reported in bovines. For this purpose, 177 goats and 248 sheep (20 percent of herds) were randomly sampled on four farms in the State of Paraíba in May and October 2008. The animals were screened for trypanosomes by the buffy coat technique (BCT) and PCR. Infected animals, approximately 25 percent in both surveys, manifested apathy, pale mucous membranes, enlarged lymph nodes, weakness, weight loss, opacity of the cornea, blindness and abortion. However, the animals with acute and severe disease showing the highest levels of parasitaemia and fever, which many times resulted in death, were only detected in the first survey. These severely diseased animals exhibited progressive weight loss and had the smallest packed cell volume (PCV) values. During the second survey done in October 2008 on the same farms, only animals with low parasitaemia and normal temperatures, PCV values and body weights were detected. Therefore, animals that spontaneously recovered from acute infection developed chronic and asymptomatic disease. This finding demonstrated for the first time that sheep and goats, which are the most important livestock in the semi-arid region of Brazil, may be severely affected by *T. vivax* infection and also play a role as asymptomatic carriers and important sources of *T. vivax* to ruminants in general.

15036. **Cabrera, L., De Witte, J., Victor, B., Vermeiren, L., Zimic, M., Brandt, J. & Geysen, D., 2009.** Specific detection and identification of African trypanosomes in bovine peripheral blood by means of a PCR-ELISA assay. *Veterinary Parasitology*, **164** (2-4): 111-117.

Alexander von Humboldt Institute of Tropical Medicine (IMTAvH),
Universidad Peruana Cayetano Heredia, Av. Honorio Delgado 430, San Martin
de Porras, Lima 31, Peru.

The aim of the present study was to develop a PCR-ELISA assay for the detection and differentiation of the main African pathogen trypanosomal species present in peripheral blood of cattle. The proposed methodology allows to specifically differentiate *Trypanosoma congolense*, *Trypanosoma vivax* and the subgenus *Trypanozoon*, by means of a sensitive universal PCR amplifying trypanosome DNA followed by an ELISA-based hybridization with three highly specific probes. The semi-nested PCR had a sensitivity of 15 fg, 15 fg, and 0.15 fg of DNA from *T. vivax*, *T. congolense*, and *Trypanosoma brucei brucei*, respectively that is sufficient to detect parasites in blood during the chronic phase of the disease. Biotinylated second round asymmetric PCR amplification products were used in an ELISA set up using three species-specific probes for the diagnosis of *T. congolense* (type Riverine, Kilifi or Savannah), *T. vivax* and *T. brucei brucei*. A factor O.D. of 0.082 was determined on blood samples from bovines (n=18) from a non-endemic area in Africa. In a pilot study of blood samples of naturally and experimentally *Trypanosoma* infected cattle previously characterized by PCR-RFLP (n=42), a high rate of concordance (93.3 percent) was found between PCR-RFLP and PCR-ELISA. There is a good ratio between positive and negative O.D. values (3.00 vs. 0.1) and the technique can also be used to distinguish mixed infections.

15037. **Da Silva, A. S., Pierezan, F., Wolkmer, P., Costa, M. M., Oliveira, C. B., Tonin, A. A., Santurio, J. M., Lopes, S. T. & Monteiro, S. G., 2009.** Pathological findings associated with experimental infection by *Trypanosoma evansi* in cats. *Journal of Comparative Pathology*, **142** (2-3): 170-176.

Department of Microbiology and Parasitology, Universidade Federal de Santa
Maria, Santa Maria, RS, Brazil. [aleksandro_ss@yahoo.com.br].

Five cats were experimentally inoculated with *Trypanosoma evansi* in order to evaluate the pathological changes induced by this protozoan infection. Clinical signs observed included vomiting, diarrhoea, hyperthermia, weight loss, facial oedema, corneal opacity, lymphadenopathy and hindlimb instability. Reduction in haematocrit was observed from seven days post-infection (dpi) ($P < 0.05$). One cat died at 40 dpi and the other four cats were humanely destroyed. Necropsy examination was performed on two cats at 56 dpi and two cats at 120 dpi. Gross findings in all cats included generalized muscle atrophy, pale mucosae, icterus of the subcutaneous and serosal tissue and the intima of arteries, lymphadenopathy and splenomegaly. Other findings included corneal opacity, subcutaneous oedema (mainly of the head) and hydropericardium. Trypomastigotes of *T. evansi* were observed in impression smears prepared from the aqueous humor. Microscopically, there was lymphoid hyperplasia of the spleen and lymph nodes. The animals with corneal opacity had mild corneal oedema and accumulation of fibrin and inflammatory cells (neutrophils and plasma cells) in the

anterior chamber. Similar inflammatory cells infiltrated the iris, ciliary body, corneoscleral limbus and conjunctiva.

15038. **Huson, L. E., Authie, E., Boulange, A. F., Goldring, J. P. & Coetzer, T. H., 2009.** Modulation of the immunogenicity of the *Trypanosoma congolense* cysteine protease, congopain, through complexation with alpha(2)-macroglobulin. *Veterinary Research*, **40** (6): 52.

School of Biochemistry, Genetics and Microbiology, University of KwaZulu-Natal (Pietermaritzburg campus), Private Bag X01, Scottsville, 3209, South Africa.

The protozoan parasite *Trypanosoma congolense* is the main causative agent of livestock trypanosomosis. Congopain, the major lysosomal cysteine proteinase of *T. congolense*, contributes to disease pathogenesis, and antibody-mediated inhibition of this enzyme may contribute to mechanisms of trypanotolerance. The potential of different adjuvants to facilitate the production of antibodies that would inhibit congopain activity was evaluated in the present study. Rabbits were immunized with the recombinant catalytic domain of congopain (C2), either without adjuvant, with Freund's adjuvant or complexed with bovine or rabbit alpha(2)-macroglobulin (alpha(2)M). The antibodies were assessed for inhibition of congopain activity. Rabbits immunized with C2 alone produced barely detectable anti-C2 antibody levels and these antibodies had no effect on recombinant C2 or native congopain activity. Rabbits immunized with C2 and Freund's adjuvant produced the highest levels of anti-C2 antibodies. These antibodies either inhibited C2 and native congopain activity to a small degree, or enhanced their activity, depending on time of production after initial immunisation. Rabbits receiving C2-alpha(2)M complexes produced moderate levels of anti-C2 antibodies and these antibodies consistently showed the best inhibition of C2 and native congopain activity of all the antibodies, with maximum inhibition of 65 percent. Results of this study suggest that antibodies inhibiting congopain activity could be raised in livestock with a congopain catalytic domain-alpha(2)M complex. This approach improves the effectiveness of the antigen as an anti-disease vaccine candidate for African trypanosomosis.

15039. **Masumu, J., Marcotty, T., Geerts, S., Vercruyse, J. & Van den Bossche, P., 2009.** Cross-protection between *Trypanosoma congolense* strains of low and high virulence. *Veterinary Parasitology*, **163** (1-2): 127-131.

University of Pretoria, Department of Veterinary Tropical Diseases, Onderstepoort, South Africa.

The aim of this study was to assess the existence of possible cross-protection between *Trypanosoma congolense* strains of low and extreme virulence circulating in the same trypanosomosis focus. Groups of six mice were infected using one of three strains of low virulence and challenged with one of three strains of extreme virulence. A group of six mice was used as control for each strain of low and extreme virulence. The results showed that mice infected with one of the strains of extreme virulence developed high parasitaemia and a significant drop of the PCV compared with mice infected with a strain of low virulence and challenged with one of the strains of extreme virulence. With an exception of one strain of

extreme virulence (strain F), the survival time of mice infected with the strains of extreme virulence was shorter compared with mice infected with strains of low virulence and subsequently challenged with a strain of extreme virulence. These results suggest that in an area where trypanosomes of various virulence profiles circulate, livestock infected with *T. congolense* strains of low virulence can be protected against the adverse effects of extremely virulent *T. congolense* strains.

(c) TRYPANOTOLERANCE

[See also 32: 15058].

15040. **Gautier, M., Flori, L., Riebler, A., Jaffrezic, F., Laloe, D., Gut, I., Moazami-Goudarzi, K. & Foulley, J. L., 2009.** A whole genome Bayesian scan for adaptive genetic divergence in West African cattle. *BMC Genomics*, **10**: 550.

INRA, UMR de Génétique Animale et Biologie Intégrative, 78350 Jouy-en-Josas, France. [Mathieu.Gautier@jouy.inra.fr].

The recent settlement of cattle in West Africa after several waves of migration from remote centres of domestication has imposed dramatic changes in their environmental conditions, in particular through exposure to new pathogens. West African cattle populations thus represent an appealing model to unravel the genome response to adaptation to tropical conditions. The purpose of this study was to identify footprints of adaptive selection at the whole genome level in a newly collected data set comprising 36 320 SNPs genotyped in nine West African cattle populations. After a detailed analysis of population structure, we performed a scan for SNP differentiation via a previously proposed Bayesian procedure including extensions to improve the detection of loci under selection. Based on these results we identified 53 genomic regions and 42 strong candidate genes. Their physiological functions were mainly related to immune response (MHC region which was found under strong balancing selection, CD79A, CXCR4, DLK1, RFX3, SEMA4A, TICAM1 and TRIM21), nervous system (NEUROD6, OLFM2, MAG11, SEMA4A and HTR4) and skin and hair properties (EDNRB, TRSP1 and KRTAP8-1). It is concluded that the main possible underlying selective pressures may be related to climatic conditions but also to the host response to pathogens such as *Trypanosoma* spp. Overall, these results might open the way towards the identification of important variants involved in adaptation to tropical conditions and in particular to resistance to tropical infectious diseases.

15041. **Maichomo, M. W., Kosura, W. O., Gathuma, J. M., Gitau, G. K., Ndung'u, J. M. & Nyamwaro, S. O., 2009.** Economic assessment of the performance of trypanotolerant cattle breeds in a pastoral production system in Kenya. *Journal of the South African Veterinary Association*, **80** (3): 157-162.

Trypanosomiasis Research Centre, Kenya Agricultural Research Institute, PO Box 362, Kikuyu, Kenya. [maichomo@yahoo.com].

Cattle are the major source of food security and income for pastoral farmers in sub-Saharan Africa. However, infectious and parasitic diseases remain a major constraint to improved cattle productivity in the region. The use of animal health economics to support

decision-making on cost-effective disease control options is increasingly becoming important in the developing world. Trypanotolerant indigenous Orma/Zebu cattle in a trypanosomosis-endemic area of Kenya were evaluated for economic performance using gross-margin analysis and partial-farm budgeting. Orma/Zebu and Sahiwal/Zebu cross-bred cattle were exposed to similar husbandry practices and monitored for growth rate, incidence of common infections (trypanosomosis, anaplasmosis, babesiosis, East Coast Fever and helminthosis) and the cost of treatment assessed. Interview questionnaires were also used to assess the preference rating of the two breeds. Results indicated that incidence of infection was trypanosomosis 3 percent, anaplasmosis 58 percent, babesiosis 11 percent, East Coast Fever 22 percent and helminthosis 28 percent, with no significant difference between breeds. The Orma/Zebu and Sahiwal/Zebu breeds had comparable economic benefits, hence a pastoralist in Magadi division is likely to get similar returns from both breeds. This study therefore recommends adoption of not only the Sahiwal/Zebu but also the Orma/Zebu breed for cattle improvement in trypanosomosis endemic areas and conservation of indigenous genetic resources.

15042. **Meade, K. G., O’Gorman, G. M., Hill, E. W., Narciandi, F., Agaba, M., Kemp, S. J., O’Farrelly, C. & MacHugh, D. E., 2009.** Divergent antimicrobial peptide (AMP) and acute phase protein (APP) responses to *Trypanosoma congolense* infection in trypanotolerant and trypanosusceptible cattle. *Molecular Immunology*, **47** (2-3): 196-204.

Animal Bioscience Centre, Teagasc, Grange, Co. Meath, Ireland.

African animal trypanosomosis (AAT) is endemic across Sub-Saharan African and is a major constraint to livestock production. The ability of certain cattle breeds to remain productive despite infection is known as trypanotolerance; however, the underlying immune mechanisms contributing to this trait remain poorly understood. Antimicrobial peptides (AMPs) and acute phase proteins (APPs) are evolutionarily conserved effector molecules of the innate immune system that have important roles in the resolution of infection and activation of the adaptive immune response. Expression levels of AMP genes (TAP, LAP, BNBD4, DEFB1, DEFB5 and LEAP2) and APP genes (HP, CP, AGP, LBP, SAA3 and CRP) were investigated using real time quantitative reverse transcription PCR (qRT-PCR) in peripheral blood mononuclear cells (PBMC) isolated from two breeds of African cattle (trypanotolerant N'Dama and trypanosusceptible Boran), experimentally infected with *Trypanosoma congolense*. Haptoglobin and serum amyloid A (SAA) were also measured in plasma using quantitative protein assays. Results demonstrated that tracheal antimicrobial peptide (TAP) gene expression increased by 32-fold in Boran, compared with only 3-fold in N'Dama, by 14 days post-infection (dpi) and rising to 136-fold at 29 dpi in Boran, compared to 47-fold in N'Dama ($P < 0.05$). Protein expression levels of SAA are elevated in N'Dama, rising to 163 $\mu\text{g/ml}$ at 14 dpi compared with 72 $\mu\text{g/ml}$ in Boran. The SAA expression profile mirrors the wave of parasitaemia detected in N'Dama. Seven single nucleotide polymorphisms (SNPs) were identified in the promoter regions of the SAA3 and SAA4 genes, which are predicted to affect transcription factor binding and thereby contributing to the differential patterns of expression detected between the breeds. Whereas elevated TAP expression is a conserved component of the innate immune response to infection in both breeds, higher SAA expression levels may contribute toward trypanotolerance in N'Dama.

15043. **O’Gorman, G. M., Park, S. D., Hill, E. W., Meade, K. G., Coussens, P. M., Agaba, M., Naessens, J., Kemp, S. J. & MacHugh, D. E., 2009.** Transcriptional profiling of cattle infected with *Trypanosoma congolense* highlights gene expression signatures underlying trypanotolerance and trypanosusceptibility. *BMC Genomics*, **10**: 207.

Animal Genomics Laboratory, UCD School of Agriculture, Food Science and Veterinary Medicine, UCD College of Life Sciences, University College Dublin, Belfield, Dublin 4, Ireland. [grace.ogorman@ucd.ie].

African animal trypanosomosis (AAT) caused by tsetse fly-transmitted protozoa of the genus *Trypanosoma* is a major constraint on livestock and agricultural production in Africa and is among the top ten global cattle diseases impacting on the poor. Here we show that a functional genomics approach can be used to identify temporal changes in host peripheral blood mononuclear cell (PBMC) gene expression due to disease progression. We also show that major gene expression differences exist between cattle from trypanotolerant and trypanosusceptible breeds. Using bovine long oligonucleotide microarrays and real time quantitative reverse transcription PCR (qRT-PCR) validation we analysed PBMC gene expression in naive trypanotolerant and trypanosusceptible cattle experimentally challenged with *Trypanosoma congolense* across a 34-day infection time course. Trypanotolerant N’Dama cattle displayed a rapid and distinct transcriptional response to infection, with a ten-fold higher number of genes differentially expressed at day 14 post-infection compared with trypanosusceptible Boran cattle. These analyses identified coordinated temporal gene expression changes for both breeds in response to trypanosome infection. In addition, a panel of genes were identified that showed pronounced differences in gene expression between the two breeds, which may underlie the phenomena of trypanotolerance and trypanosusceptibility. Gene ontology (GO) analysis demonstrate that the products of these genes may contribute to increased mitochondrial mRNA translational efficiency, a more pronounced B cell response, an elevated activation status and a heightened response to stress in trypanotolerant cattle. This study has revealed an extensive and diverse range of cellular processes that are altered temporally in response to trypanosome infection in African cattle. Results indicate that the trypanotolerant N’Dama cattle respond more rapidly and with a greater magnitude to infection compared with the trypanosusceptible Boran cattle. Specifically, a subset of the genes analysed by real time qRT-PCR, which display significant breed differences, could collectively contribute to the trypanotolerance trait in N’Dama.

(d) TREATMENT

[See also **32**: 15050].

15044. **Grace, D., Randolph, T., Affognon, H., Dramane, D., Diall, O. & Clausen, P. H., 2009.** Characterization and validation of farmers' knowledge and practice of cattle trypanosomosis management in the cotton zone of West Africa. *Acta Tropica*, **111** (2): 137-143.

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

We carried out a knowledge, attitude, practice (KAP) survey on how farmers (n=895) manage cattle trypanosomosis in Burkina Faso, Mali and Guinea. Most farmers (96 percent) recognized the common signs of trypanosomosis, 70 percent knew the role of tsetse flies in transmitting the disease and 96 percent had knowledge of drugs used for treatment. Farmers reported that trypanosomosis was the most important cattle disease and estimated that 25 percent of their herd fall sick each year and 18 percent of the sick animals die. Nearly all sick animals (90 percent) were treated with trypanocides and most treatments were administered by untrained farmers. Giving drugs was the strategy most used as primary means of protection (50 percent of farmers) followed by avoiding high risk areas (32 percent of farmers) and keeping trypanotolerant cattle (7 percent of farmers). Few farmers knew about communal tsetse control methods and those who did, rarely practised them. Farmer diagnosis of trypanosomosis in cattle presented at clinics (n=113) was in most cases (84 percent) supported by laboratory tests. However, the signs that most farmers considered indicative of trypanosomosis (staring coat and emaciation) were poor predictors of trypanosomosis. We tested farmer knowledge of injection sites and trypanocide dilutions (n=423 cattle), and while few (15 percent) farmers gave under-dosages or over-dosage (2 percent of farmers), injection techniques were poor with injection-related side effects in 24 percent of cattle treated by farmers. Despite this, therapeutic outcomes were both objectively (clinical parameters) and subjectively (carer assessment) satisfactory in 89 percent of cattle treated by farmers. This study found that farmers play a major role in successfully managing trypanosomosis and recommends the recognition and support for community based treatment.

15045. **Mbaya, A. W., Aliyu, M. M., Nwosu, C. O., Taiwo, V. O. & Ibrahim, U. I., 2009.** Effects of melarsamine hydrochloride (Cymelarsan) and diminazene aceturate (Berenil) on the pathology of experimental *Trypanosoma brucei* infection in red fronted gazelles (*Gazella rufifrons*). *Veterinary Parasitology*, **163** (1-2): 140-143.

Department of Veterinary Microbiology & Parasitology, University of Maiduguri, Nigeria. [awmbaya@yahoo.com].

An experimental infection of red fronted gazelles (*Gazella rufifrons*) with *Trypanosoma brucei* strain MKAR/84/NITR/6 was carried out. Two waves of parasitaemia which corresponded with a significant decline ($P<0.05$) in packed cell volume (PCV) were encountered in the infected untreated controls and those treated at day 8 post-infection with a sub-optimal dosage of diminazene aceturate (Berenil) at 3.5 mg/kg body weight. At postmortem, hepatomegally, splenomegally, lymphadenopathy, nephritis, myocardial degeneration with pulmonary oedema were observed in the two groups. Similarly, histopathological studies of some organs revealed interstitial haemorrhages, severe degenerative changes with cellular infiltrations. On the other hand, those treated by day eight post-infection with melarsamine hydrochloride (Cymelarsan) at 0.3 mg/kg, 0.6 mg/kg or diminazene aceturate (Berenil) at 7.0 mg/kg body weight had apparently normal organs at the end of the experiment. These results suggest that *T. brucei* can cause severe pathological changes in untreated red fronted gazelles (*Gazella rufifrons*). However, treatments at the onset of parasitaemia, by day eight post-infection with melarsamine hydrochloride (Cymelarsan) at 0.3 and 0.6 mg/kg or diminazene aceturate (Berenil) at 7.0 mg/kg body weight ameliorated the deleterious effects of the infection in the gazelles.

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

(b) PATHOLOGY AND IMMUNOLOGY

[See also **32**: 15039, 15109, 15137, 15143, 15161, 15163, 15187].

15046. **Antoine-Moussiaux, N., Cornet, A., Cornet, F., Glineur, S., Dermine, M. & Desmecht, D., 2009.** A non-cytosolic protein of *Trypanosoma evansi* induces CD45-dependent lymphocyte death. *PLoS One*, **4** (5): e5728.

Department of Pathology, Faculty of Veterinary Medicine, University of Liège, Liège, Belgium.

In a recent study dealing with a mouse model of *Trypanosoma evansi*-associated disease, a remarkable synchrony between the parasitaemia peak and the white-blood-cell count nadir was noticed. The present study was designed to establish whether there is a direct causal link between the parasite load during its exponential phase of growth and the disappearance of peripheral blood leukocytes. *In vitro* experiments performed with trypanosomes and purified peripheral blood mononucleated cells revealed the existence of a lymphotoxin embedded in the *T. evansi* membrane: a protein sensitive to serine proteases, with a molecular mass of less than 30 kDa. Lymphocytes death induced by this protein was found to depend on the intervention of a lymphocytic protein tyrosine phosphatase. When lymphocytes were exposed to increasing quantities of a monoclonal antibody raised against the extracellular portion of CD45, a transmembrane protein tyrosine phosphatase covering over 10 percent of the lymphocyte surface, *T. evansi* membrane extracts showed a dose-dependent decrease in cytotoxicity. As the regulatory functions of CD45 concern not only the fate of lymphocytes but also the activation threshold of the TCR-dependent signal and the amplitude and nature of cytokinetic effects, this demonstration of its involvement in *T. evansi*-dependent lymphotoxicity suggests that *T. evansi* might manipulate, via CD45, the host's cytokinetic and adaptive responses.

15047. **Barry, D. & McCulloch, R., 2009.** Molecular microbiology: A key event in survival. *Nature*, **459** (7244): 172-173.

Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow G12 8TA, UK. [j.d.barry@bio.gla.ac.uk].

This paper deals with the mechanisms underpinning antigenic variation in *T. brucei* – the periodical switching of its variant surface glycoprotein (VSG) which is the molecule targeted by the hosts humoral immune response. They describe the essentials of a paper written by Boothroyd et al. appearing in the same issue of this journal dealing with how switching is triggered – the evidence presented being that a DNA double-stranded break (DSB) upstream of the *T. brucei* VSG gene is the likely primary event. They point out that the findings of Boothroyd et al suggest a model for switching in which natural breaks occur in the active expression site which precipitate conversion repair from another locus that contains a distinct but inactive VSG. This then raises the question of how breaks occur – by

an endonuclease?, by a DNA modification repair process? by transcription –associated breakage? by unstable repeats stalling DNA replication, creating DSB-like free DMNA ends that stimulate DNA repair through recombination mechanisms?.

15048. **Boschaerts, T., Guilliams, M., Stijlemans, B., De Baetselier, P. & Beschin, A., 2009.** Understanding the role of monocytic cells in liver inflammation using parasite infection as a model. *Immunobiology*, **214** (9-10): 737-747.

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

Uncontrolled inflammation is a major cause of pathogenicity during chronic parasite infections. Novel therapies should therefore aim at re-establishing the balance between pro- and anti-inflammatory signals during disease to avoid tissue damage and ensure survival of the host. In this context, we are intending to identify strategies capable of inducing counter-inflammatory activity in injured liver and thereby increasing the resistance of the host to African trypanosomiasis as a model for parasite infection. Here, recent evidence is summarized revealing how monocytic cells recruited to the liver of African trypanosome-infected mice develop an M1 or M2 activation status, thereby maintaining the capacity of the host to control parasite growth while avoiding the development of liver damage, which otherwise culminates in early death of the host.

15049. **Dagenais, T. R., Freeman, B. E., Demick, K. P., Paulnock, D. M. & Mansfield, J. M., 2009.** Processing and presentation of variant surface glycoprotein molecules to T cells in African trypanosomiasis. *Journal of Immunology*, **183** (5): 3344-3355.

Department of Bacteriology, University of Wisconsin-Madison, Madison, WI 53706, USA.

Th1 cell responses to the variant surface glycoprotein (VSG) of African trypanosomes play a critical role in controlling infection through the production of IFN-gamma, but the role of APCs in the induction and regulation of T cell-mediated protection is poorly understood. In this study, we have investigated the Ag presentation capabilities of dendritic cells (DCs) and macrophages during early trypanosome infection in relatively resistant responder and susceptible nonresponder mouse strains. Splenic DCs appeared to be the primary cell responsible for activating naive VSG-specific Th cell responses in resistant responder animals through the coordinated up-regulation of costimulatory molecules, secretion of IL-12, and presentation of VSG peptides to T cells *in vivo*. Splenic DC depletion and the down-regulation of costimulatory markers on splenic macrophages were observed in susceptible animals and may be associated with the inability of these animals to elicit a significant VSG-specific T cell response. In contrast to splenic APCs, peritoneal macrophages secreted NO, failed to activate naive Th cells *in vitro*, and presented relatively low levels of VSG peptides to T cells *in vivo*. Thus, VSG-specific Th1 cell responses may be determined by tissue- and cell-specific differences in Ag presentation. Additionally, all APCs from resistant and susceptible strains displayed a reduced ability to process and present newly encountered exogenous Ag, including new VSG molecules, during high parasitaemia. Thus, initial uptake of VSG (or other trypanosome factors) may interfere with Ag presentation and have dramatic consequences for subsequent T cell responses to other proteins.

15050. **Fon-tebug, S., Maganga, G., Gbati, O., Pangui, L., Awah-ndukum, J., & Keambou, T., 2009.** Effects of co-administration of vitamin B12 with diminazene aceturate on packed cell volume and weight gain in cattle experimentally infected with *Trypanosoma congolense*. *International Journal of Biological and Chemical Sciences*, **3** (6): 1219-1225.

Ecole Inter-Etats des Sciences et Médecine Vétérinaires (EISMV), P.O. Box 5077 Dakar, Sénégal; University of Dschang, Faculty of Agronomy and Agricultural Science (FASA), P.O.Box 222 Dschang, Cameroon. [fon2tebug@yahoo.com / st_fon@hotmail.com].

The effects of co-administration of cyanocobalamin and/or hydroxocobalamin with diminazene aceturate (DA) on pack cell volume and weight gain in cattle experimentally infected with *Trypanosoma congolense* were studied. Twenty eight young zebu bulls aged 10-16 months with an average weight of 92.02 ± 14.74 kg were randomly distributed into 4 groups. These bulls were infected with *Trypanosoma congolense* intravenously at a dose rate of 1×10^5 suspended in 4 ml of phosphate buffered saline per animal. Each group was treated with a commercial medication containing DA, cyanocobalamin and/or hydroxocobalamin 10 d post-infection. Haematological examination showed no trypanosomes irrespective of the regimen administered 48 h post-treatment in all infected cattle. Packed Cell Volume (PCV) and weight gain were highest with the regimen containing DA, cyanocobalamin and hydroxocobalamin. The trypanocidal regimens containing DA co-administered with cyanocobalamin and/or hydroxocobalamin enabled a rapid reconstitution of red blood cells and led to improvement in the weight gain of the trypanosome-infected cattle.

15051. **Gibellini, F., Hunter, W. N. & Smith, T. K., 2009.** The ethanolamine branch of the Kennedy pathway is essential in the bloodstream form of *Trypanosoma brucei*. *Molecular Microbiology*, **73** (5): 826-843.

Division of Biological Chemistry and Drug Discovery, School of Life Sciences, University of Dundee, Dundee, Scotland, UK.

Phosphatidylethanolamine (GPEtn), a major phospholipid component of trypanosome membranes, is synthesized *de novo* from ethanolamine through the Kennedy pathway. Here the composition of the GPEtn molecular species in the bloodstream form of *Trypanosoma brucei* is determined, along with new insights into phospholipid metabolism, by *in vitro* and *in vivo* characterization of a key enzyme of the Kennedy pathway, the cytosolic ethanolamine-phosphate cytidyltransferase (TbECT). Gene knockout indicates that TbECT is essential for growth and survival, thus highlighting the importance of the Kennedy pathway for the pathogenic stage of the African trypanosome. Phosphatidylserine decarboxylation, a potential salvage pathway, does not appear to be active in cultured bloodstream form *T. brucei*, and it is not upregulated even when the Kennedy pathway is disrupted. *In vivo* metabolic labelling and phospholipid composition analysis by ESI-MS/MS of the knockout cells confirmed a significant decrease in GPEtn species, as well as changes in the relative abundance of other phospholipid species. Reduction in GPEtn levels had a profound influence on the morphology of the mutants and it compromised mitochondrial structure and

function, as well as glycosylphosphatidylinositol anchor biosynthesis. TbECT is therefore genetically validated as a potential drug target against the African trypanosome.

15052. **Giroud, C., Ottones, F., Coustou, V., Dacheux, D., Biteau, N., Miezan, B., Van Reet, N., Carrington, M., Doua, F. & Baltz, T., 2009.** Murine models for *Trypanosoma brucei gambiense* disease progression-from silent to chronic infections and early brain tropism. *PLoS Neglected Tropical Diseases*, **3** (9): e509.

UMR 5234, Centre National de Recherche Scientifique, IFR66, Université Bordeaux 2, Bordeaux, France.

Human African trypanosomosis (HAT) caused by *Trypanosoma brucei gambiense* remains highly prevalent in west and central Africa and is lethal if left untreated. The major problem is that the disease often evolves toward chronic or asymptomatic forms with low and fluctuating parasitaemia producing apparently aparasitaemic serological suspects who remain untreated because of the toxicity of the chemotherapy. Whether the different types of infections are due to host or parasite factors has been difficult to address, since *T. b. gambiense* isolated from patients is often not infectious in rodents thus limiting the variety of isolates. *T. b. gambiense* parasites were outgrown directly from the cerebrospinal fluid of infected patients by *in vitro* culture and analysed for their molecular polymorphisms. Experimental murine infections showed that these isolates could be clustered into three groups with different characteristics regarding their *in vivo* infection properties, immune response and capacity for brain invasion. The first isolate induced a classical chronic infection with a fluctuating blood parasitaemia, an invasion of the central nervous system (CNS), a trypanosome specific-antibody response and death of the animals within 6-8 months. The second group induced a sub-chronic infection resulting in a single wave of parasitaemia after infection, followed by a low parasitaemia with no parasites detected by microscope observations of blood but detected by PCR, and the presence of a specific antibody response. The third isolate induced a silent infection characterized by the absence of microscopically detectable parasites throughout, but infection was detectable by PCR during the whole course of infection. Additionally, specific antibodies were barely detectable when mice were infected with a low number of this group of parasites. In both sub-chronic and chronic infections, most of the mice survived more than one year without major clinical symptoms despite an early dissemination and growth of the parasites in different organs including the CNS, as demonstrated by bioluminescent imaging. Whereas trypanosome characterization assigned all these isolates to the homogeneous Group I of *T. b. gambiense*, they clearly induce very different infections in mice thus mimicking the broad clinical diversity observed in HAT due to *T. b. gambiense*. Therefore, these murine models will be very useful for the understanding of different aspects of the physiopathology of HAT and for the development of new diagnostic tools and drugs.

15053. **Gomez-Rodriguez, J., Stijlemans, B., De Muylder, G., Korf, H., Brys, L., Berberof, M., Darji, A., Pays, E., De Baetselier, P. & Beschin, A., 2009.** Identification of a parasitic immunomodulatory protein triggering the development of suppressive M1 macrophages during African trypanosomiasis. *Journal of Infectious Diseases*, **200** (12): 1849-1860.

Department of Molecular and Cellular Interactions, Vlaams Institute voor Biotechnologie (VIB), Vrije Universiteit Brussel, 1050 Brussels, Belgium.

Development of classically activated macrophages (M1 cells) is a prerequisite to controlling parasite growth and therefore resistance to African trypanosomosis. However, if activation of M1 cells is uncontrolled, including their production of tumour necrosis factor (TNF) and nitric oxide (NO), collateral pathogenic damage to tissues ensues. We report the identification of a novel putative *Trypanosoma brucei* M1 cell-triggering protein. The recombinant trypanosome-suppressive immunomodulating factor (rTSIF) induced TNF and NO secretion by macrophages. Moreover, M1 cells triggered by rTSIF block T cell proliferation in a manner dependent on NO, interferon gamma, and cell contact. Furthermore, rTSIF could down-regulate type 2-oriented immune responses. Therefore, trypanosome-suppressive immunomodulating factor (TSIF) may represent a new parasite molecule with the potential to modulate the host immune network, whereby it could contribute to the inflammatory response required to control parasite growth and to the pathogenicity of African trypanosomosis, including immunosuppression. TSIF knock-down trypanosomes died within two days, indicating that TSIF may be essential for parasite biology.

15054. **Herbas, M. S., Thekiso, O. M., Inoue, N., Xuan, X., Arai, H. & Suzuki, H., 2009.**

The effect of alpha-tocopherol transfer protein gene disruption on *Trypanosoma congolense* infection in mice. *Free Radical Biology and Medicine*, **47** (10): 1408-1413.

Research Unit for Functional Genomics, National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Nishi 2-13, Inada, Obihiro 080-8555, Japan.

At present 15 to 20 million people are estimated to be infected with pathogenic trypanosome parasites worldwide, mainly in developing countries. There are a number of factors that affect the severity of trypanosomosis, including the nutritional status of the host. However, the relationship between micronutrient levels and trypanosomosis outcome has yet to be reported in detail. Here, we demonstrate that the inhibition of alpha-tocopherol transfer protein, a determinant of the vitamin E concentration in host circulation, confers resistance to *Trypanosoma congolense* infection, evidently owing to oxidative damage to parasite DNA. These results suggest that transient inhibition of alpha-tocopherol transfer gene activity could possibly be exploited as a strategy for both the prevention and the treatment of trypanosomosis.

15055. **Landeira, D., Bart, J. M., Van Tyne, D. & Navarro, M., 2009.** Cohesin regulates VSG monoallelic expression in trypanosomes. *Journal of Cell Biology*, **186** (2): 243-254.

Instituto de Parasitología y Biomedicina Lopez-Neyra, Consejo Superior de Investigaciones Científicas, 18100 Granada, Spain.

Antigenic variation allows *Trypanosoma brucei* to evade the host immune response by switching the expression of one out of approximately 15 telomeric variant surface glycoprotein (VSG) expression sites (ESs). VSG ES transcription is mediated by RNA

polymerase I in a discrete nuclear site named the ES body (ESB). However, nothing is known about how the monoallelic VSG ES transcriptional state is maintained over generations. In this study, we show that during S and G2 phases and early mitosis, the active VSG ES locus remains associated with the single ESB and exhibits a delay in the separation of sister chromatids relative to control loci. This delay is dependent on the cohesin complex, as partial knockdown of cohesin subunits resulted in premature separation of sister chromatids of the active VSG ES. Cohesin depletion also prompted transcriptional switching from the active to previously inactive VSG ESs. Thus, in addition to maintaining sister chromatid cohesion during mitosis, the cohesin complex plays an essential role in the correct epigenetic inheritance of the active transcriptional VSG ES state.

15056. **Lecordier, L., Vanhollebeke, B., Poelvoorde, P., Tebabi, P., Paturiaux-Hanocq, F., Andris, F., Lins, L. & Pays, E., 2009.** C-terminal mutants of apolipoprotein L-I efficiently kill both *Trypanosoma brucei brucei* and *Trypanosoma brucei rhodesiense*. *PLoS Pathogens*, **5** (12): e1000685.

Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, Gosselies, Belgium.

Apolipoprotein L-I (apoL1) is a human-specific serum protein that kills *Trypanosoma brucei* through ionic pore formation in endosomal membranes of the parasite. The *T. brucei* subspecies *rhodesiense* and *gambiense* resist this lytic activity and can infect humans, causing sleeping sickness. In the case of *T. b. rhodesiense*, resistance to lysis involves interaction of the serum resistance-associated (SRA) protein with the C-terminal helix of apoL1. We undertook a mutational and deletional analysis of the C-terminal helix of apoL1 to investigate the linkage between interaction with SRA and lytic potential for different *T. brucei* subspecies. We confirm that the C-terminal helix is the SRA-interacting domain. Although in *E. coli* this domain was dispensable for ionic pore-forming activity, its interaction with SRA resulted in inhibition of this activity. Different mutations affecting the C-terminal helix reduced the interaction of apoL1 with SRA. However, mutants in the L370-L392 leucine zipper also lost *in vitro* trypanolytic activity. Truncating and/or mutating the C-terminal sequence of human apoL1 like that of apoL1-like sequences of *Papio anubis* resulted in both loss of interaction with SRA and acquired ability to efficiently kill human serum-resistant *T. b. rhodesiense* parasites, *in vitro* as well as in transgenic mice. These findings demonstrate that SRA interaction with the C-terminal helix of apoL1 inhibits its pore-forming activity and determines resistance of *T. b. rhodesiense* to human serum. In addition, they provide a possible explanation for the ability of *Papio* serum to kill *T. b. rhodesiense*, and offer a perspective to generate transgenic cattle resistant to both *T. b. brucei* and *T. b. rhodesiense*.

15057. **Mishra, B. B., Gundra, U. M. & Teale, J. M., 2009.** Toll-like receptors in CNS parasitic infections. *Current Topics in Microbiology and Immunology*, **336**: 83-104.

Department of Biology, South Texas Center for Emerging Infectious Diseases, The University of Texas at San Antonio, One UTSA Circle, San Antonio, TX, 78249-1644, USA.

Parasite infections in the central nervous system (CNS) are a major cause of morbidity and mortality worldwide, second only to HIV infection. Finding appropriate therapeutic measures to control CNS parasite infections requires an understanding of the tissue-specific host response. CNS parasitic diseases are invariably associated with persistent T-helper 1 (Th1) cytokine-dependent proinflammatory responses. Although type 1 cytokine-dependent proinflammatory responses are essential to control several types of parasite infections, their persistent production contributes to the development of neuropathology with severe consequences. A family of proteins called Toll-like receptors (TLRs) plays a pivotal role in the induction of inflammatory cytokines during infections and tissue injury. Accumulating evidence indicates that in several CNS parasitic infections such as toxoplasmosis and sleeping sickness, host responses mediated through TLRs contribute to parasite clearance and host survival. However, TLR-mediated responses can also contribute to disease severity, as exemplified in cerebral malaria, neurocysticercosis and river blindness. Thus, TLRs influence the immunopathogenesis of CNS parasitic infections by mechanisms that can either benefit the host or further contribute to CNS pathology. This chapter discusses the immunopathogenesis of parasitic infections in the CNS and the role of TLRs in this process.

15058. **Namangala, B., & Beschin, A., 2009.** Quantitative differences in immune responses in mouse strains that differ in their susceptibility to *Trypanosoma brucei brucei* infection. *Journal of Veterinary Medical Science*, **71** (7): 951-956.

The University of Zambia, Department of Paraclinical Studies, Lusaka, Zambia.
[boniface_1020@yahoo.com].

We compared the relative resistance and soluble variant surface glycoprotein (VSG)-specific responses in (C57BL/6 x BALB/c)-F1 (B6B-F1) and C3H mice during infection with *Trypanosoma brucei brucei*, the haemoprotozoan parasite causing a debilitating disease in man and livestock. We demonstrated that C3H mice are relatively more trypanosusceptible, as evidenced by their reduced ability to control parasitaemia and shorter survival time, than B6B-F1 mice. Quantitative differences in the pattern of cytokine and antibody (Ab) production were observed between the two mouse strains following infection with *T. b. brucei*. Thus, although both mouse strains recorded detectable levels of IFN-gamma, TNF-alpha, NO and IL-10 in plasma and lymph nodes, as well as plasma IgM, IgG1, IgG2a, IgG2b and IgG3 Abs against VSG, the susceptible C3H mice only exhibited trace levels of Abs of all isotypes and yet produced elevated levels of IFN-gamma, TNF-alpha and NO, compared to the relatively trypanotolerant B6B-F1 mice. In aggregate, these data strongly suggest that trypanosome-infected C3H mice have an immunological defect, manifested not only by suppression at the B cell clonal level, but also at the level of protective T cell and macrophage phenotypes.

15059. **Ngotho, M., Kagira, J. M., Jensen, H. E., Karanja, S. M., Farah, I. O. & Hau, J., 2009.** Immunospecific immunoglobulins and IL-10 as markers for *Trypanosoma brucei rhodesiense* late stage disease in experimentally infected vervet monkeys. *Tropical Medicine and International Health*, **14** (7): 736-747.

Institute of Primate Research, Karen, Nairobi, Kenya.

To determine the usefulness of IL-10 and immunoglobulin M (IgM) as biomarkers for staging HAT in vervet monkeys, a useful pathogenesis model for humans, vervet monkeys were infected with *Trypanosoma brucei rhodesiense* and subsequently given sub-curative and curative treatment 28 and 140 days post-infection (dpi) respectively. Matched serum and CSF samples were obtained at regular intervals and immunospecific IgM, immunoglobulin G (IgG) and IL-10 were quantified by ELISA. There was no detectable immunospecific IgM and IgG in the CSF before 49 dpi. CSF white cell counts, IgM and IgG, and serum IgM were significantly elevated with peak levels coinciding with meningoencephalitis 98 dpi. The serum IL-10 was upregulated in both early and late disease stage, coinciding with primary and relapse parasitaemia respectively. CSF white cell counts were elevated progressively until curative treatment was given. After curative treatment, there was rapid and significant drop in serum IgM and IL-10 concentrations as well as CSF white cell counts. However, the CSF IgM and IgG remained detectable to the end of the study. It is concluded that serum and CSF concentrations of immunospecific IgM and CSF IgG changes followed a pattern that mimics the progression of the disease and may present reliable and useful biomarkers of the disease stage. Due to rapid decline, serum IgM and IL-10 are, additionally, potential biomarkers of the success of chemotherapy.

15060. **Ngure, R. M., Eckersall, P., Burke, J., Karori, S. M., Mwangi, W. W., Wachira, F. N., Maathai, R. & Murray, M., 2009.** Endotoxin-like effects in acute phase response to *Trypanosoma brucei brucei* infection are not due to gastrointestinal leakage. *Parasitology International*, **58** (4): 325-329.

Department of Biochemistry and Molecular Biology, Egerton University, Egerton, Kenya. [ramuch68@yahoo.com].

Trypanosomosis is mainly an immunological and inflammatory response mediated by increased levels of pro-inflammatory cytokines. Evidence suggests that pathological changes produced during infection with trypanosomes could be initiated by nonspecific endotoxin-like substances in trypanosomes and/or Gram-negative secondary bacterial infection. Studies in trypanosome-infected rats indicate damage to the gastrointestinal tract (GIT) accompanied by increased leakage of the GIT mucosa. The current study was carried out to determine the *in vivo* response to endotoxin-like substances of *Trypanosoma brucei brucei*. For this purpose we neutralized the entrance of endotoxin through the GIT using polymyxin-B treatment and monitored the plasma concentration of the acute phase proteins SAP and Hp. The results of this study, where infection was performed in the presence of oral antibiotic that is not absorbed from GIT and which binds to and inactivates endotoxin, show that the elevated plasma levels of endotoxin-like activity and the resulting acute phase response indicated by an increase in levels of Hp and SAP, are due to trypanosome infection. Results obtained in the present study indicate that GIT is not the major source of elevated plasma endotoxin-like activity levels and the observed acute phase response was due to an increase in the levels of acute phase proteins SAP and haptoglobin. Therefore trypanosomes are responsible for the elevated plasma endotoxin-like activity levels and the subsequent systemic acute phase response in the host.

15061. **Nielsen, M. J. & Moestrup, S. K., 2009.** Receptor targeting of haemoglobin mediated by the haptoglobins: roles beyond heme scavenging. *Blood*, **114** (4): 764-771.

Department of Medical Biochemistry, University of Aarhus, Aarhus, Denmark.

Haptoglobin, the haptoglobin-haemoglobin receptor CD163, and the haeme oxygenase-1 are proteins with a well-established function in the clearance and metabolism of "free" haemoglobin released during intravascular haemolysis. This scavenging system counteracts the potentially harmful oxidative and NO-scavenging effects associated with "free" haemoglobin, and, furthermore, elicits an anti-inflammatory response. In the late primate evolution, haptoglobin variants with distinct functions have arisen, including haptoglobin polymers and the haptoglobin-related protein. The latter associates with a subspecies of high-density lipoprotein (HDL) particles that play a crucial role in the innate immunity against certain trypanosome parasites. Recent studies have elucidated this fairly sophisticated immune defence mechanism that takes advantage of a trypanosomal haptoglobin-haemoglobin receptor evolved to supply the parasite with haeme. Because of the high resemblance between haptoglobin and haptoglobin-related protein, the receptor also takes up the complex of haemoglobin and the HDL-bound haptoglobin-related protein. This tricks the parasite into internalizing another HDL-associated protein and toxin, apolipoprotein L-I, that kills the parasite. In conclusion, variant human homologous haemoglobin-binding proteins that collectively may be designated the haptoglobins have diverted from the haptoglobin gene. On haemoglobin and receptor interaction, these haptoglobins contribute to different biologic events that go beyond simple removal from plasma of the toxic haemoglobin.

15062. **Nishimura, K., Yanase, T., Nakagawa, H., Matsuo, S., Ohnishi, Y. & Yamasaki, S., 2009.** Effect of polyamine-deficient chow on *Trypanosoma brucei brucei* infection in rats. *Journal of Parasitology*, **95** (4): 781-786.

Laboratory of Infectious Diseases Control, Graduate School of Life and Environmental Sciences, Osaka Prefecture University, Sakai, Osaka 599-8531, Japan. [nishimura@vet.osakafu-u.ac.jp].

Polyamines are essential for proliferation of *Trypanosoma brucei brucei*, and feeding rats polyamine-deficient chow (PDC) decreases their blood polyamine concentrations. Proliferation of *T. b. brucei* (IL-tat 1.4 strain) (IL) is not restrained within PDC-fed rats. However, symptoms of IL-infected rats such as anaemia decrease by PDC feeding. We reported cytokine and nitric oxide (NO) production by *T. b. gambiense* (Wellcome strain [WS])-infected rats were affected by PDC feeding, and WS proliferation was restrained. Therefore, we investigated whether the change in production of cytokines and NO by PDC feeding affects IL proliferation and decreases symptoms *in vivo*. In IL-infected PDC-fed rats, NO, interleukin (IL)-12, and tumour necrosis factor-alpha production increased while interferon-gamma and IL-10 decreased compared with normal chow-fed rats. IL proliferation was restrained by NO production when it was co-cultured with spleen cells harvested from uninfected rats. In contrast, IL proliferation in infected rats was not changed by PDC feeding, although NO production was increased. The results suggest that changes in cytokines and NO production in IL-infected rats by PDC feeding have little influence on IL proliferation. However, they may serve to decrease symptoms.

15063. **Okwor, I., Muleme, H., Jia, P. & Uzonna, J. E., 2009.** Altered proinflammatory cytokine production and enhanced resistance to *Trypanosoma congolense* infection

in lymphotoxin beta-deficient mice. *Journal of Infectious Diseases*, **200** (3): 361-369.

Department of Immunology, Faculty of Medicine, University of Manitoba, Winnipeg, Canada.

BALB/c mice are highly susceptible to *Trypanosoma congolense* infection, whereas C57BL/6 mice are relatively resistant. Overproduction of interferon-gamma (IFN-gamma) and other proinflammatory cytokines contribute to death in susceptible mice. Here, we show that lymphotoxin beta-deficient (LTbeta(-/-)) mice are more resistant than wild-type (WT) mice to *T. congolense* infection, as shown by a lower parasitaemia levels and a longer survival duration. The enhanced resistance of LTbeta(-/-) mice was associated with undetectable or low serum levels of proinflammatory cytokines (i.e., tumour necrosis factor-alpha, interleukin [IL]-6, IL-12, and monocyte chemoattractant protein-1). Although infected LTbeta(-/-) mice had high numbers of CD4(+)CD25(+)Foxp3(+) cells and high serum IL-10 levels, these cells were not the major producers of IL-10. Treatment of LTbeta(-/-) mice with anti-IL-10R monoclonal antibody abolished their enhanced resistance, whereas depletion of CD25(+) cells further enhanced resistance among infected WT and LTbeta(-/-) mice. These results suggest that LTbeta plays critical role in regulating the outcome of *T. congolense* infection in mice.

15064. Rathkolb, B., Noyes, H. A., Brass, A., Dark, P., Fuchs, H., Gailus-Durner, V., Gibson, J., de Angelis, M. H., Ogugo, M., Iraqi, F., Kemp, S. J., Naessens, J., Pope, M. E., Wolf, E. & Agaba, M., 2009. Clinical chemistry of congenic mice with quantitative trait loci for predicted responses to *Trypanosoma congolense* infection. *Infection and Immunity*, **77** (9): 3948-3957.

International Livestock Research Institute, Nairobi 00100, Kenya.

Trypanosoma congolense is a protozoan parasite that causes severe diseases in livestock. Three major quantitative trait loci (QTL), Tir1, Tir2, and Tir3, control the survival time of mice after infection with *T. congolense*. Congenic mice carrying the C57BL/6 resistance alleles on the A/J background were developed for each of these loci. The congenic mice were used to physically map the regions containing the QTL gene(s) and to investigate the physiological effect of each locus. Clinical chemistry data for infected A/J, C57BL/6, and BALB/c mice were obtained for 15 analytes at five time points. Congenic mice were assessed for survival, parasitaemia, and anaemia as well as seven clinical-chemical analytes. The survival times were significantly increased in the Tir1 and Tir2 mice but not Tir3 congenic mice. The survival time of the parental inbred mice correlated negatively with parasitaemia but positively with alanine aminotransferase activities in serum, suggesting that inflammatory reactions in the liver had a beneficial effect possibly associated with reduced parasitaemia. However, there was no difference in parasitaemia or liver enzyme activities of Tir1 and Tir2 congenic mice relative to their controls, showing that survival, parasitaemia, and degree of liver damage are not associated with each other, despite the correlation in the parental lines. These data suggest that the congenic loci affect survival but do not affect control of parasite number. They may therefore act by limiting the pathological consequences of *T. congolense* infection.

15065. **Silva, M. S., Prazeres, D. M., Lanca, A., Atouguia, J. & Monteiro, G. A., 2009.** Trans-sialidase from *Trypanosoma brucei* as a potential target for DNA vaccine development against African trypanosomiasis. *Parasitology Research*, **105** (5): 1223-1229.

Unidade de Ensino e Investigacao de Clinica das Doencas Tropicais, Centro de Malaria e Outras Doencas Tropicais, Instituto de Higiene e Medicina Tropical, 1349-008 Lisboa, Portugal. [mssilva@ihmt.unl.pt].

African trypanosomosis (AT), also known as sleeping sickness in humans and Nagana in animals, is a disease caused by the protozoan parasite *Trypanosoma brucei*. AT is an extremely debilitating disease in human, cattle, and wild animals, and the treatment is difficult with frequent relapses. This work shows that BALB-c mice immunized intramuscularly with a single dose (100 µg) of a plasmid DNA encoding the 5'-terminal region of the trans-sialidase (nTSA) gene of *T. brucei brucei* are able to produce IgG antibodies that bind to the bloodstream form of *T. brucei brucei*-protein extract and recognize the recombinant nTSA protein, expressed in *Escherichia coli*. Furthermore, this DNA vaccination process was able to protect 60 percent of mice submitted to a challenge with the infective form of *T. brucei brucei* parasites. These results demonstrate that a DNA vaccine coding for trans-sialidase from *T. brucei* is potentially useful in the prophylaxis of AT.

15066. **Smith, T. K., Vasileva, N., Gluenz, E., Terry, S., Portman, N., Kramer, S., Carrington, M., Michaeli, S., Gull, K. & Rudenko, G., 2009.** Blocking variant surface glycoprotein synthesis in *Trypanosoma brucei* triggers a general arrest in translation initiation. *PLoS One*, **4** (10): e7532.

Centre for Biomolecular Sciences, University of St. Andrews, Fife, Scotland, UK.

The African trypanosome *Trypanosoma brucei* is covered with a dense layer of variant surface glycoprotein (VSG), which protects it from lysis by host complement via the alternative pathway in the mammalian bloodstream. Blocking VSG synthesis by the induction of VSG RNAi triggers an unusually precise precytokinesis cell-cycle arrest. Here, we characterize the cells arrested after the induction of VSG RNAi. We were able to rescue the VSG221 RNAi induced cell-cycle arrest through expression of a second different VSG (VSG117 which is not recognized by the VSG221 RNAi) from the VSG221 expression site. Metabolic labeling of the arrested cells showed that blocking VSG synthesis triggered a global translation arrest, with total protein synthesis reduced to less than 1-4 percent of normal levels within 24 h of induction of VSG RNAi. Analysis by electron microscopy showed that the translation arrest was coupled with rapid disassociation of ribosomes from the endoplasmic reticulum. Polysome analysis showed a drastic decrease in polysomes in the arrested cells. No major changes were found in levels of transcription, total RNA transcript levels or global amino acid concentrations in the arrested cells. In conclusion, the cell-cycle arrest phenotype triggered by the induction of VSG221 RNAi is not caused by siRNA toxicity, as this arrest can be alleviated if a second different VSG is inserted downstream of the active VSG221 expression site promoter. Analysis of polysomes in the stalled cells showed that the translation arrest is mediated at the level of translation initiation rather than elongation. The cell-cycle arrest induced in the presence of a VSG synthesis block is

reversible, suggesting that VSG synthesis and/or trafficking to the cell surface could be monitored during the cell-cycle as part of a specific cell-cycle checkpoint.

15067. **Taiwo, V. O., Anosa, V. O. & Oluwaniyi, J. O., 2000.** Differential expression of surface membrane antigens on bovine monocytes activated with recombinant cytokines and during *Trypanosoma congolense* infection. *Onderstepoort Journal of Veterinary Research*, **67** (4): 289-296.

Department of Veterinary Pathology, University of Ibadan, Nigeria.

The expression of surface membrane antigens on peripheral blood monocytes (PBM) of cattle of the Boran and N'Dama breeds activated with recombinant cytokines (TNF-alpha and IFN-gamma) and during experimental infection with *Trypanosoma congolense* was investigated using monoclonal antibodies (MoAbs) and fluorescein-activated cell sorter (FACS). The surface antigens investigated were C3bi receptor, major histocompatibility (MHC) II complex (Ia antigen) and two monocyte/macrophage (Mphi) differentiation antigens. The study revealed that both cytokines caused the enhancement of the expression of all the PBM surface antigens studied. rBoIFN-gamma at low concentrations was more efficient in causing the activation of PBM. While the PBM of Boran cattle were more significantly activated to express the C3bi receptor vis-a-vis the Ia antigen than N'Dama cattle, the reverse was the case with the PBM of N'Dama cattle which expressed more Ia antigens than Boran PBM. Similar results were observed during *T. congolense* infection in the two breeds of cattle. The significantly higher expression of C3bi receptor and correspondingly lower Ia antigen expression by the PBM of Boran cattle, both during trypanosomosis and *in vitro* may be responsible for the higher rate of erythrocyte phagocytosis, hence the development of more severe anaemia by Boran cattle during trypanosomosis than N'Dama. In addition, the expression of significantly higher numbers of Ia antigen by N'Dama Mphi better enables N'Dama to process, present and initiate a better trypanosome antigen-specific immune response than Boran cattle during infection. These two attributes are known genetic characteristics of trypanotolerance in cattle.

15068. **Wolkmer, P., da Silva, A. S., Traesel, C. K., Paim, F. C., Cargnelutti, J. F., Pagnoncelli, M., Picada, M. E., Monteiro, S. G. & Lopes, S. T., 2009.** Lipid peroxidation associated with anemia in rats experimentally infected with *Trypanosoma evansi*. *Veterinary Parasitology*, **165** (1-2): 41-46.

Laboratory of Veterinary Clinical Analysis--LACVet, Federal University of Santa Maria, Santa Maria, RS, Brazil. [patiwol@hotmail.com].

This study aimed to assess the plasma lipid peroxidation and the susceptibility of erythrocytes to *in vitro* peroxidation as indicators of oxidative damage in erythrocytes and their roles in the pathogenesis of anaemia during the early acute phase of *Trypanosoma evansi* infection in rats. Fifty male Wistar rats were randomly distributed into seven groups: three trypanosome-infected groups (T(2), T(4) and T(6); n=10 animals per group) and four uninfected controls (C(0), C(2), C(4) and C(6); n=5 animals per group). Animals from trypanosome-infected groups were inoculated intraperitoneally with 10^6 trypanosomes. Blood samples were collected by cardiac puncture before infection (day 0; group C(0)) or on the 2nd (C(2) and T(2)), 4th (C(4) and T(4)) and 6th (C(6) and T(6)) days post-infection (dpi). Samples

were analysed for red blood cell (RBC) count, haemoglobin (Hb) concentration, packed cell volume (PCV), plasma malondialdehyde (MDA) and *in vitro* peroxidation of erythrocytes. The mean values of the haematological indices gradually decreased in the infected rats compared with the control. MDA was significantly increased ($P < 0.001$) on the 6th dpi in infected versus control animals and was negatively correlated with PCV ($P < 0.001$; $R^2 = 0.372$). The values for erythrocyte *in vitro* peroxidation were higher for groups T(4) and T(6) than for the control rats ($P < 0.01$). A positive correlation between erythrocyte peroxidation and MDA ($P < 0.001$; $R^2 = 0.414$) was observed. The results of this study indicate that *T. evansi* infection in rats is associated with oxidative stress, indicated by lipid peroxidation and oxidative damage in erythrocyte membranes, as demonstrated by *in vitro* peroxidation. This may be one of the causes of anaemia in acute trypanosomosis.

(c) CHEMOTHERAPEUTICS

15069. **Altenkamper, M., Bechem, B., Perruchon, J., Heinrich, S., Madel, A., Ortman, R., Dahse, H. M., Freunscht, E., Wang, Y., Rath, J., Stich, A., Hitzler, M., Chiba, P., Lanzer, M. & Schlitzer, M., 2009.** Antimalarial and antitrypanosomal activity of a series of amide and sulfonamide derivatives of a 2,5-diaminobenzophenone. *Bioorganic and Medicinal Chemistry*, **17** (22): 7690-7697.

Institut für Pharmazeutische Chemie, Philipps-Universität Marburg, Marbacher Weg 6, 35032 Marburg, Germany.

15070. **Bacchi, C. J., Barker, R. H., Jr., Rodriguez, A., Hirth, B., Rattendi, D., Yarlett, N., Hendrick, C. L. & Sybertz, E., 2009.** Trypanocidal activity of 8-methyl-5'-{[(Z)-4-aminobut-2-enyl]-(methylamino)}adenosine (Genz-644131), an adenosylmethionine decarboxylase inhibitor. *Antimicrobial Agents and Chemotherapy*, **53** (8): 3269-3272.

Haskins Laboratories, Pace University, 41 Park Row, New York, NY 10038, USA. [cbacchi@pace.edu]

Genzyme 644131, 8-methyl-5'-{[(Z)-4-aminobut-2-enyl]-(methylamino)}adenosine, is an analogue of the enzyme activated S-adenosylmethionine decarboxylase (AdoMetDC) inhibitor and the trypanocidal agent MDL-7381, 5'-{[(Z)-4-aminobut-2-enyl]-(methylamino)}adenosine. The analogue differs from the parent in having an 8-methyl group on the purine ring that bestows favourable pharmacokinetic, biochemical, and trypanocidal activities. The compound was curative in acute *Trypanosoma brucei brucei* and drug-resistant *Trypanosoma brucei rhodesiense* model infections, with single-dose activity in the 1- to 5-mg/kg/day daily dose range for four days against *T. brucei brucei* and 25- to 50-mg/kg twice-daily dosing against *T. brucei rhodesiense* infections. The compound was not curative in the TREU 667 central nervous system model infection but cleared blood parasitaemia and extended time to recrudescence in several groups. This study shows that AdoMetDC remains an attractive chemotherapeutic target in African trypanosomes and that chemical changes in AdoMetDC inhibitors can produce more favourable drug characteristics than the lead compound.

15071. **Bakunova, S. M., Bakunov, S. A., Wenzler, T., Barszcz, T., Werbovets, K. A., Brun, R. & Tidwell, R. R., 2009.** Synthesis and antiprotozoal activity of pyridyl analogues of pentamidine. *Journal of Medicinal Chemistry*, **52** (15): 4657-4667.

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

A series of novel pyridyl analogues 1-18 of the antiprotozoal drug 1,5-bis(4-amidinophenoxy)pentane (pentamidine) has been synthesized and tested for *in vitro* activities against *Trypanosoma brucei rhodesiense*, *Plasmodium falciparum*, and *Leishmania donovani*, and for cytotoxicity against mammalian cells. Antiprotozoal properties of compounds 1-18 depended on the placement of cationic moieties on the pyridine rings as well as the nature of substituents on the amidine groups. Diamidine 6 with cationic moieties adjacent to pyridine nitrogen atoms was the most promising compound in the series showing superior *in vitro* activities against *T. brucei rhodesiense*, *P. falciparum*, and *L. donovani* compared with pentamidine. An oral prodrug of diamidine 6, diamidoxime 9, administered at 25 mg/kg daily for four days, exhibited excellent antitrypanosomal efficacy *in vivo* curing all infected animals in the STIB900 acute mouse model of trypanosomosis.

15072. **Bawm, S., Tiwananthagorn, S., Lin, K. S., Hirota, J., Irie, T., Htun, L. L., Maw, N. N., Myaing, T. T., Phay, N., Miyazaki, S., Sakurai, T., Oku, Y., Matsuura, H. & Katakura, K., 2009.** Evaluation of Myanmar medicinal plant extracts for antitrypanosomal and cytotoxic activities. *Journal of Veterinary Medical Science*. e **Publication ahead of print, December 22.**

Laboratory of Parasitology, Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Japan.

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

15073. **Bryant, C., Kerr, I. D., Debnath, M., Ang, K. K., Ratnam, J., Ferreira, R. S., Jaishankar, P., Zhao, D., Arkin, M. R., McKerrow, J. H., Brinen, L. S. & Renslo, A. R., 2009.** Novel non-peptidic vinylsulfones targeting the S2 and S3 subsites of parasite cysteine proteases. *Bioorganic and Medicinal Chemistry Letters*, **19** (21): 6218-6221.

Small Molecule Discovery Center, University of California, San Francisco, CA 94158, USA.

15074. **Cavalli, A. & Bolognesi, M. L., 2009.** Neglected tropical diseases: multi-targeted ligands in the search for novel lead candidates against *Trypanosoma* and *Leishmania*. *J. Medicinal Chemistry*, **52** (23): 7339-7359.

Department of Pharmaceutical Sciences, Alma Mater Studiorum, University of Bologna, Via Belmeloro 6, 40126 Bologna, Italy.

No abstract available

15075. **Collar, C. J., Al-Salabi, M. I., Stewart, M. L., Barrett, M. P., Wilson, W. D. & de Koning, H. P., 2009.** Predictive computational models of substrate binding by a nucleoside transporter. *Journal of Biological Chemistry*, **284** (49): 34028-34035.

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

Transporters play a vital role in both the resistance mechanisms of existing drugs and effective targeting of their replacements. Melarsoprol and diamidine compounds similar to pentamidine and furamidine are primarily taken up by trypanosomes of the genus *Trypanosoma brucei* through the P2 aminopurine transporter. In standardized competition experiments with ³H adenosine, P2 transporter inhibition constants (K_i) have been determined for a diverse dataset of adenosine analogues, diamidines, Food and Drug Administration-approved compounds and analogues thereof, and custom-designed trypanocidal compounds. Computational biology has been employed to investigate compound structure diversity in relation to P2 transporter interaction. These explorations have led to models for inhibition predictions of known and novel compounds to obtain information about the molecular basis for P2 transporter inhibition. A common pharmacophore for P2 transporter inhibition has been identified along with other key structural characteristics. Our model provides insight into P2 transporter interactions with known compounds and contributes to strategies for the design of novel antiparasitic compounds. This approach offers a quantitative and predictive tool for molecular recognition by specific transporters without the need for structural or even primary sequence information of the transport protein.

15076. **Dardonville, C., Fernandez-Fernandez, C., Gibbons, S. L., Jagerovic, N., Nieto, L., Ryan, G., Kaiser, M. & Brun, R., 2009.** Antiprotozoal activity of 1-phenethyl-4-aminopiperidine derivatives. *Antimicrobial Agents and Chemotherapy*, **53** (9): 3815-3821.

Instituto de Quimica Medica, CSIC, Juan de la Cierva 3, E-28006 Madrid, Spain. [dardonville@iqm.csic.es].

A series of 44 4-aminopiperidine derivatives was screened *in vitro* against four protozoan parasites (*Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani*, and *Plasmodium falciparum*). This screening identified 29 molecules selectively active against bloodstream-form *T. b. rhodesiense* trypomastigotes, with 50 percent inhibitory concentrations (IC₅₀) ranging from 0.12 to 10 μM, and 33 compounds active against the chloroquine- and pyrimethamine-resistant K1 strain of *P. falciparum* (IC₅₀ range, 0.17 to 5 μM). In addition, seven compounds displayed activity against intracellular *T. cruzi*

amastigotes in the same range as the reference drug benznidazole (IC₅₀, 1.97 μ M) but were also cytotoxic to L-6 cells, showing little selectivity for *T. cruzi*. None of the molecules tested showed interesting antileishmanial activity against axenic amastigotes of *L. donovani*. To our knowledge, this is the first report of the antitrypanosomal activity of molecules bearing the 4-aminopiperidine skeleton.

15077. **Dube, A., Gupta, R. & Singh, N., 2009.** Reporter genes facilitating discovery of drugs targeting protozoan parasites. *Trends in Parasitology*, **25** (9): 432-439.

Division of Parasitology, Central Drug Research Institute, Lucknow 226 001, India. [anuradha_dube@hotmail.com].

Transfection of protozoan parasites, such as *Plasmodium*, *Leishmania*, *Trypanosoma* and *Toxoplasma*, with various reporter gene constructs, has revolutionized studies to understand the biology of the host-parasite interactions at the cellular level. It has provided impetus to the development of rapid and reliable drug screens both for established drugs and for new molecules against different parasites and other pathogens. Furthermore, reporter genes have proved to be an excellent and promising tool for studying disease progression. Here, we review the recent advances made by using reporter genes for *in vitro* and *in vivo* drug screening, high-throughput screening, whole-animal non-invasive imaging for parasites and for the study of several aspects of host-parasite interactions.

15078. **Eberle, C., Burkhard, J. A., Stump, B., Kaiser, M., Brun, R., Krauth-Siegel, R. L. & Diederich, F., 2009.** Synthesis, inhibition potency, binding mode, and antiprotozoal activities of fluorescent inhibitors of trypanothione reductase based on mepacrine-conjugated diaryl sulfide scaffolds. *ChemMedChem*, **4** (12): 2034-2044.

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

Trypanothione reductase (TR) is a flavoenzyme unique to trypanosomatid parasites and a target for lead discovery programs. Various inhibitor scaffolds have emerged in the past, exhibiting moderate affinity for the parasite enzyme. Herein we show that the combination of two structural motifs of known TR inhibitors - diaryl sulfides and mepacrine - enables the simultaneous addressing of two hydrophobic patches in the active site. The binding efficacy of these conjugates is enhanced over that of the respective parent inhibitors. They show K_(ic) values for the parasite enzyme down to 0.9 \pm 0.1 μ M and exhibit high selectivity for TR over human glutathione reductase (GR). Despite their considerable molecular mass and in some cases permanent positive charges, *in vitro* studies revealed IC₅₀ values in the low micromolar to sub-micromolar range against *Trypanosoma brucei rhodesiense* and *Trypanosoma cruzi*, as well as the malaria parasite *Plasmodium falciparum*, which lack trypanothione metabolism. The inhibitors exhibit strong fluorescence due to their aminoacridine moiety. This feature allows visualization of the drugs in the parasite where high accumulation was observed by fluorescence microscopy even after short exposure times.

15079. **Gigante, F., Kaiser, M., Brun, R. & Gilbert, I. H., 2009.** SAR studies on azasterols as potential anti-trypanosomal and anti-leishmanial agents. *Bioorganic and Medicinal Chemistry*, **17** (16): 5950-5961.

University of Dundee, Sir James Black Centre, UK.

15080. **Gillingwater, K., Kumar, A., Anbazhagan, M., Boykin, D. W., Tidwell, R. R. & Brun, R., 2009.** *In vivo* investigations of selected diamidine compounds against *Trypanosoma evansi* using a mouse model. *Antimicrobial Agents and Chemotherapy*, **53** (12): 5074-5079.

Parasite Chemotherapy, Department of Medical Parasitology and Infection Biology, Swiss Tropical Institute, Basel, Switzerland.

Surra is an animal pathogenic protozoan infection, caused by *Trypanosoma evansi*, that develops into a fatal wasting disease. Control measures rely on diagnosis and treatment. However, with the continuous emergence of drug resistance, this tactic is failing, and the pressing need for new chemotherapeutic agents is becoming critical. With the introduction of novel aromatic diamidines, a new category of antitrypanosomal drugs was discovered. Nevertheless, their efficacy within a *T. evansi*-infected mouse model was not known. In total, 30 compounds previously selected based on their *in vitro* activity were tested in a *T. evansi* mouse model of infection. Six of the compounds were capable of curing *T. evansi*-infected mice at drug doses as low as 0.5 and 0.25 mg/kg of body weight administered for four consecutive days, and they were more effective than the standard drugs suramin, diminazene, and quinapyramine. After all selection criteria were applied, three diamidine compounds (DB 75, DB 867, and DB 1192) qualified as lead compounds and were considered to have the potential to act as preclinical candidates against *T. evansi* infection.

15081. **Hirth, B., Barker, R. H., Jr., Celatka, C. A., Klinger, J. D., Liu, H., Nare, B., Nijjar, A., Phillips, M. A., Sybertz, E., Willert, E. K. & Xiang, Y., 2009.** Discovery of new S-adenosylmethionine decarboxylase inhibitors for the treatment of Human African Trypanosomiasis (HAT). *Bioorganic and Medicinal Chemistry Letters*, **19** (11): 2916-2919.

Drug and Biomaterial R&D, Genzyme Corporation, Waltham, MA 02451, USA.

Modification of the structure of trypanosomal AdoMetDC inhibitor 1 (MDL73811) resulted in the identification of a new inhibitor 7a, which features a methyl substituent at the 8-position. Compound 7a exhibits improved potencies against both the trypanosomal AdoMetDC enzyme and parasites, and better blood brain barrier penetration than inhibitor 1.

15082. **Hu, L., Arafa, R. K., Ismail, M. A., Patel, A., Munde, M., Wilson, W. D., Wenzler, T., Brun, R. & Boykin, D. W., 2009.** Synthesis and activity of azaterphenyl diamidines against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum*. *Bioorganic and Medicinal Chemistry*, **17** (18): 6651-6658.

Department of Chemistry, Georgia State University, Atlanta, GA 30303-3083, USA.

A series of azaterphenyl diamidines has been synthesized and evaluated for *in vitro* antiprotozoal activity against both *Trypanosoma brucei rhodesiense* (*T. b. r.*) and *Plasmodium falciparum* (*P. f.*) and *in vivo* efficacy in the STIB900 acute mouse model for *T. b. r.* Six of the 13 compounds showed IC₅₀ values less than 7 nM against *T. b. r.* Twelve of those exhibited IC₅₀ values less than 6 nM against *P. f.* and six of those showed IC₅₀ values 0.6 nM, which are more than 25-fold as potent as furamidine. Moreover, two of them showed more than 40-fold selectivity for *P. f.* versus *T. b. r.* Three compounds 15b, 19d and 19e exhibited *in vivo* efficacy against *T. b. r.* much superior to furamidine, and equivalent to or better than azafuramidine. The antiparasitic activity of these diamidines depends on the ring nitrogen atom(s) location relative to the amidine groups and generally correlates with DNA binding affinity.

15083. **Huang, T. L., Vanden Eynde, J. J., Mayence, A., Collins, M. S., Cushion, M. T., Rattendi, D., Londono, I., Mazumder, L., Bacchi, C. J. & Yarleth, N., 2009.** Synthesis and SAR of alkanediamide-linked bisbenzamidines with anti-trypanosomal and anti-pneumocystis activity. *Bioorganic and Medicinal Chemistry Letters*, **19** (20): 5884-5886.

Xavier University of Louisiana, College of Pharmacy, 1 Drexel Drive, New Orleans, LA 70125, USA. [thuang@xula.edu].

15084. **Kerr, I. D., Lee, J. H., Farady, C. J., Marion, R., Rickert, M., Sajid, M., Pandey, K. C., Caffrey, C. R., Legac, J., Hansell, E., McKerrow, J. H., Craik, C. S., Rosenthal, P. J. & Brinen, L. S., 2009.** Vinyl sulfones as antiparasitic agents and a structural basis for drug design. *Journal of Biological Chemistry*, **284** (38): 25697-25703.

Department of Cellular and Molecular Pharmacology, University of California, San Francisco, California 94158-2550, USA.

Cysteine proteases of the papain superfamily are implicated in a number of cellular processes and are important virulence factors in the pathogenesis of parasitic disease. These enzymes have therefore emerged as promising targets for antiparasitic drugs. We report the crystal structures of three major parasite cysteine proteases, cruzain, falcipain-3, and the first reported structure of rhodesain, in complex with a class of potent, small molecule, cysteine protease inhibitors, the vinyl sulfones. These data, in conjunction with comparative inhibition kinetics, provide insight into the molecular mechanisms that drive cysteine protease inhibition by vinyl sulfones, the binding specificity of these important proteases and the potential of vinyl sulfones as antiparasitic drugs.

15085. **Mallari, J. P., Shelat, A. A., Kosinski, A., Caffrey, C. R., Connelly, M., Zhu, F., McKerrow, J. H. & Guy, R. K., 2009.** Structure-guided development of selective Tbc1B inhibitors. *Journal of Medical Chemistry*, **52** (20): 6489-6493.

Graduate Program in Chemistry and Chemical Biology, University of California, San Francisco, California 94143-2280, USA.

The trypanosomal cathepsin TbcatB is essential for parasite survival and is an attractive therapeutic target. Herein we report the structure-guided development of TbcatB inhibitors with specificity relative to rhodesain and human cathepsins B and L. Inhibitors were tested for enzymatic activity, trypanocidal activity, and general cytotoxicity. These data chemically validate TbcatB as a drug target and demonstrate that it is possible to potently and selectively inhibit TbcatB relative to trypanosomal and human homologues.

15086. **Mina, J. G., Pan, S. Y., Wansadhipathi, N. K., Bruce, C. R., Shams-Eldin, H., Schwarz, R. T., Steel, P. G. & Denny, P. W., 2009.** The *Trypanosoma brucei* sphingolipid synthase, an essential enzyme and drug target. *Molecular and Biochemical Parasitology*, **168** (1): 16-23.

Centre for Bioactive Chemistry, Department of Chemistry and School of Biological and Biomedical Sciences, Durham University, Durham, UK.

Sphingolipids are important components of eukaryotic membranes, particularly the plasma membrane, and are involved in a diverse array of signal transduction processes. In the Eukaryota the biosynthetic pathway for the formation of these lipid species is largely conserved. However, in contrast to mammals which produce sphingomyelin (SM), several pathogenic fungi and protozoa synthesize inositol phosphorylceramide (IPC) as the primary phosphosphingolipid. This process is catalyzed by the enzyme IPC synthase, a recognized target for anti-fungals encoded by the AUR1 gene in yeast. Recently, functional orthologues of the AUR1p have been identified in a group of insect vector-borne pathogenic protozoa, the Kinetoplastida, which are responsible for a range of so-called neglected diseases. Of these, the *Trypanosoma brucei* species are the causative agents of human African trypanosomosis in many of the most under-developed regions of Africa. The available treatments for these diseases are limited, of decreasing efficacy, and often demonstrate severe side-effects. Against this background the *T. brucei* sphingolipid synthase, an orthologue of the yeast AUR1p, may represent a promising target for novel anti-protozoals. Our studies identify an isoform of this protein as a novel bi-functional enzyme capable of catalyzing the synthesis of both IPC and SM, both known to be present in the parasite. Furthermore, the synthase is essential for parasite growth and can be inhibited by a known anti-fungal at low nanomolar levels *in vitro*. Most notably this drug demonstrates trypanocidal activity against cultured bloodstream form parasites. Thus, the *T. brucei* sphingolipid synthase represents a valid and promising drug target.

15087. **Mpamhanga, C. P., Spinks, D., Tulloch, L. B., Shanks, E. J., Robinson, D. A., Collie, I. T., Fairlamb, A. H., Wyatt, P. G., Frearson, J. A., Hunter, W. N., Gilbert, I. H. & Brenk, R., 2009.** One scaffold, three binding modes: novel and selective pteridine reductase 1 inhibitors derived from fragment hits discovered by virtual screening. *Journal of Medical Chemistry*, **52** (14): 4454-4465.

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

15088. **Nibret, E., Sporer, F., Asres, K. & Wink, M., 2009.** Antitrypanosomal and cytotoxic activities of pyrrolizidine alkaloid-producing plants of Ethiopia. *Journal of Pharmacy and Pharmacology*, **61** (6): 801-808.

Institut für Pharmazie und Molekulare Biotechnologie, Universität Heidelberg, im Neuenheimer Feld 364, Heidelberg, Germany.

The objective was to determine the *in-vitro* effect of extracts from 19 Ethiopian plant species and four pure pyrrolizidine alkaloids on bloodstream forms of *Trypanosoma brucei* and human leukaemia HL-60 cells. Crude plant extracts were prepared using methanol and dichloromethane. The alkaloidal extracts from *Solanecio angulatus* flowers were prepared with and without zinc reduction using the acid-base extraction method. Cell proliferation inhibitory activity of the extracts and compounds was assessed using Alamarblue. The most active extract was the dichloromethane extract of *Solanecio angulatus* flowers, with an IC₅₀ value of 12.17 µg/ml. The best selectivity index (SI > 41.08) was obtained for the same extract determined with HL-60 cells. The reduced alkaloidal extract prepared from *S. angulatus* flowers and after acid-base extraction showed more antitrypanosomal activity than unreduced alkaloidal extract with an IC₅₀ value of 14.35 µg/ml and with a selectivity index of 12.23. The second most active extract was the dichloromethane extract of *Crotalaria phillipsiae* twigs with an IC₅₀ value of 12.67 µg/ml and a selectivity index of 34.35. Most of the other extracts tested showed moderate antitrypanosomal activities to variable extents. Among the four pure pyrrolizidine alkaloids tested, senecionine showed moderate antitrypanosomal activity with an IC₅₀ value of 41.78 µg/ml. It is concluded that *Solanecio angulatus* (flowers) and *Crotalaria phillipsiae* (twigs) could serve as sources of novel trypanocidal compounds for the treatment of trypanosomosis.

15089. **Ogungbe, I. V. & Setzer, W. N., 2009.** Comparative molecular docking of antitrypanosomal natural products into multiple *Trypanosoma brucei* drug targets. *Molecules*, **14** (4): 1513-1536.

Department of Chemistry, University of Alabama in Huntsville, Huntsville, AL 35899, USA. [dayovictor2000@yahoo.com].

Antitrypanosomal natural products with different structural motifs previously shown to have growth inhibitory activity against *Trypanosoma brucei* were docked into validated drug targets of the parasite, which include trypanothione reductase, rhodesain, farnesyl diphosphate synthase, and triosephosphate isomerase. The *in-silico* calculations predicted that lowest energy docked poses of a number of the compounds can interact with catalysis-dependent residues, thus making them possible catalytic inhibitors and of course physiologically active. Compounds that possess a number of hydrogen-bond-accepting and/or -donating groups like phenolics and quinones show extensive interactions with the targets. Compounds like cissampeloflavone, 3-geranylemodin and ningpogenin thus offer profound promise.

15090. **Papanastasiou, I., Tsotinis, A., Zoidis, G., Kolocouris, N., Prathalingam, S. R. & Kelly, J. M., 2009.** Design and synthesis of *Trypanosoma brucei* active 1-alkoxy

and 1-benzyloxadamantano 2-guanylhydrazones. *ChemMedChem*, **4** (7): 1059-1062.

Faculty of Pharmacy, Department of Pharmaceutical Chemistry, University of Athens, Panepistimioupoli-Zografou, 157 71 Athens, Greece.

15091. **Pascholati, C. P., Lopera, E. P., Pavinatto, F. J., Caseli, L., Nobre, T. M., Zaniquelli, M. E., Viitala, T., D'Silva, C. & Oliveira, O. N., Jr., 2009.** The interaction of an antiparasitic peptide active against African sleeping sickness with cell membrane models. *Colloids and Surfaces B Biointerfaces*, **74** (2): 504-510.

Instituto de Fisica de Sao Carlos, Universidade de Sao Paulo, 13560-970 Sao Carlos, SP, Brazil.

Zwitterionic peptides with trypanocidal activity are promising lead compounds for the treatment of African sleeping sickness, and have motivated research into the design of compounds capable of disrupting the protozoan membrane. In this study, we use the Langmuir monolayer technique to investigate the surface properties of an antiparasitic peptide, namely S-(2,4-dinitrophenyl)glutathione di-2-propyl ester, and its interaction with a model membrane comprising a phospholipid monolayer. The drug formed stable Langmuir monolayers, whose main feature was a phase transition accompanied by a negative surface elasticity. This was attributed to aggregation upon compression due to intermolecular bond associations of the molecules, inferred from surface pressure and surface potential isotherms, Brewster angle microscopy (BAM) images, infrared spectroscopy and dynamic elasticity measurements. When co-spread with dipalmitoyl phosphatidyl choline (DPPC), the drug affected both the surface pressure and the monolayer morphology, even at high surface pressures and with low amounts of the drug. The results were interpreted by assuming a repulsive, cooperative interaction between the drug and DPPC molecules. Such repulsive interaction and the large changes in fluidity arising from drug aggregation may be related to the disruption of the membrane, which is key for the parasite killing property.

15092. **Patterson, S., Jones, D. C., Shanks, E. J., Frearson, J. A., Gilbert, I. H., Wyatt, P. G. & Fairlamb, A. H., 2009.** Synthesis and evaluation of 1-(1-(Benzo[b]thiophen-2-yl)cyclohexyl)piperidine (BTCP) analogues as inhibitors of trypanothione reductase. *ChemMedChem*, **4** (8): 1341-1353.

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

15093. **Richardson, J. L., Nett, I. R., Jones, D. C., Abdille, M. H., Gilbert, I. H. & Fairlamb, A. H., 2009.** Improved tricyclic inhibitors of trypanothione reductase by screening and chemical synthesis. *ChemMedChem*, **4** (8): 1333-1340.

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

Trypanothione reductase (TryR) is a key validated enzyme in the trypanothione-based redox metabolism of pathogenic trypanosomes and leishmania parasites. This system is

absent in humans, being replaced with glutathione and glutathione reductase, and as such offers a target for selective inhibition. As part of a programme to discover antiparasitic drugs, the LOPAC1280 library of 1 266 compounds was screened against TryR and the top hits evaluated against glutathione reductase and *T. brucei* parasites. The top hits included a number of known tricyclic neuroleptic drugs along with other new scaffolds for TryR. Three novel drug-like hits were identified and SAR studies on one of these using information from the tricyclic neuroleptic agents led to the discovery of a competitive inhibitor ($K(i)=330$ nM) with an improved potency against *T. brucei* ($EC_{50}=775$ nM).

15094. **Schmidt, T. J., Nour, A. M., Khalid, S. A., Kaiser, M. & Brun, R., 2009.** Quantitative structure-antiprotozoal activity relationships of sesquiterpene lactones. *Molecules*, **14** (6): 2062-2076.

Westfälische Wilhelms-Universität Münster, Institut für Pharmazeutische Biologie und Phytochemie, Hittorfstrasse 56, D-48149 Münster, Germany. [thomschm@uni-muenster.de].

Prompted by results of our previous studies where we found high activity of some sesquiterpene lactones (STLs) against *Trypanosoma brucei rhodesiense* (which causes East African sleeping sickness), we have now conducted a structure-(*in-vitro*)-activity study on a set of 40 STLs against *T. brucei rhodesiense*, *T. cruzi*, *Leishmania donovani* and *Plasmodium falciparum*. Furthermore, cytotoxic activity against L6 rat skeletal myoblast cells was assessed. Some of the compounds possess high activity, especially against *T. brucei* (e.g. helenalin and some of its esters with IC_{50} values of 0.05-0.1 μ M, which is about 10 times lower than their cytotoxic activity. It was found that all investigated antiprotozoal activities are significantly correlated with cytotoxicity and the major determinants for activity are a,b-unsaturated structural elements, also known to be essential for other biological activities of STLs. It was observed, however, that certain compounds are considerably more toxic against protozoa than against mammalian cells while others are more cytotoxic than active against the protozoa. A comparative QSAR analysis was therefore undertaken, in order to discern the antiparasitic activity of STLs against *T. brucei* and cytotoxicity. Both activities were found to depend to a large extent on the same structural elements and molecular properties. The observed variance in the biological data can be explained in terms of subtle variations in the relative influences of various molecular descriptors.

15095. **Schulz, M., Bakker, B. M. & Klipp, E., 2009.** Tide: a software for the systematic scanning of drug targets in kinetic network models. *BMC Bioinformatics*, **10**: 344.

Theoretical Biophysics, Institute for Biology, Humboldt Universität zu Berlin, Invalidenstrasse, 42, 10115 Berlin, Germany. [marvin.schulz@biologie.hu-berlin.de].

During the stages of the development of a potent drug candidate compounds can fail for several reasons. One of them, the efficacy of a candidate, can be estimated *in silico* if an appropriate ordinary differential equation model of the affected pathway is available. With such a model at hand it is also possible to detect reactions having a large effect on a certain variable such as a substance concentration. We show an algorithm that systematically tests the influence of activators and inhibitors of different type and strength acting at different

positions in the network. The effect on a quantity to be selected (e.g. a steady state flux or concentration) is calculated. Moreover, combinations of two inhibitors or one inhibitor and one activator targeting different network positions are analysed. Furthermore, we present TId (Target Identification), an open source, platform independent tool to investigate ordinary differential equation models in the common systems biology markup language format. It automatically assigns the respectively altered kinetics to the inhibited or activated reactions, performs the necessary calculations, and provides a graphical output of the analytical results. For illustration, TId is used to detect optimal inhibitor positions in simple branched networks, a signalling pathway, and a well studied model of glycolysis in *Trypanosoma brucei*. Using TId, we show in the branched models under which conditions inhibitions in a certain pathway can affect molecule concentrations. In the signalling pathway we illuminate which inhibitions have an effect on the signalling characteristics of the last active kinase. Finally, we compare our set of best targets in the glycolysis model with a similar analysis showing the applicability of our tool.

15096. **Spavieri, J., Kaiser, M., Casey, R., Hingley-Wilson, S., Lalvani, A., Blunden, G. & Tasdemir, D., 2009.** Antiprotozoal, antimycobacterial and cytotoxic potential of some British green algae. *Phytotherapy Research*. **e Publication ahead of print, December 3.**

Department of Pharmaceutical and Biological Chemistry, Centre for Pharmacognosy and Phytotherapy, School of Pharmacy, University of London, London WC1N 1AX, UK.

In the continuation of our search for natural sources for antiprotozoal and antitubercular molecules, we have screened the crude extracts of four green marine algae (*Cladophora rupestris*, *Codium fragile* ssp. *tomentosoides*, *Ulva intestinalis* and *Ulva lactuca*) collected from the Dorset area of England. *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, *Leishmania donovani* and *Mycobacterium tuberculosis* were used as test organisms in the *in vitro* assays. The selective toxicity of the extracts was also determined toward mammalian skeletal myoblast (L6) cells. The crude seaweed extracts had no activity against *M. tuberculosis*, but showed antiprotozoal activity against at least two protozoan species. All algal extracts were active against *T. brucei rhodesiense*, with *C. rupestris* being the most potent one (IC₅₀ value 3.7 µg/ml), whilst only *C. rupestris* and *U. lactuca* had moderate trypanocidal activity against *T. cruzi* (IC₅₀ values 80.8 and 34.9 µg/ml). Again, all four extracts showed leishmanicidal activity with IC₅₀ values ranging between 12.0 and 20.2 µg/ml. None of the extracts showed cytotoxicity toward L6 cells, indicating that their antiprotozoal activity is specific. This is the first study reporting antiprotozoal and antimycobacterial activity of British marine algae.

15097. **Spinks, D., Shanks, E. J., Cleghorn, L. A., McElroy, S., Jones, D., James, D., Fairlamb, A. H., Frearson, J. A., Wyatt, P. G. & Gilbert, I. H., 2009.** Investigation of trypanothione reductase as a drug target in *Trypanosoma brucei*. *ChemMedChem*, **4** (12): 2060-2069.

Drug Discovery Unit, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee, DD1 5EH, UK.

There is an urgent need for new drugs for the treatment of tropical parasitic diseases such as human African trypanosomosis, which is caused by *Trypanosoma brucei*. The enzyme trypanothione reductase (TryR) is a potential drug target within these organisms. Herein we report the screening of a 62 000 compound library against *T. brucei* TryR. Further work was undertaken to optimize potency and selectivity of two novel-compound series arising from the enzymatic and whole parasite screens and mammalian cell counterscreens. Both of these series, containing either a quinoline or pyrimidinopyrazine scaffold, yielded low micromolar inhibitors of the enzyme and growth of the parasite. The challenges of inhibiting TryR with drug-like molecules are discussed.

15098. **Steverding, D., Wang, X. & Sexton, D. W., 2009.** The trypanocidal effect of NO-releasing agents is not due to inhibition of the major cysteine proteinase in *Trypanosoma brucei*. *Parasitology Research*, **105** (5): 1333-1338.

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

The lysosomal cysteine proteinase activity of bloodstream forms of *Trypanosoma brucei* is a validated drug target. Previously, it was reported that nitric oxide (NO)-releasing agents inhibit the catalytic activity of cysteine proteinases of the protozoan parasites *Leishmania infantum*, *Trypanosoma cruzi* and *Plasmodium falciparum*. In this study, we investigated the effect of the NO-donors S-nitrosoglutathione, (+/-)-(E)-4-ethyl-2[(E)-hydroxyimino]-5-nitro-3-hexenamide, 3-morpholinopyridinone (SIN-1) and S-nitroso-N-acetyl-DL-penicillamine on the activity of the cysteine proteinase of *T. brucei*. At a concentration of 1 mM, the NO donors inhibited the catalytic activity of purified *T. brucei* cysteine proteinase by 50-90 percent. With the exception of SIN-1, all NO donors displayed trypanocidal activities against bloodstream forms of *T. brucei in vitro* with 50 percent growth inhibition values of around 30 μ M. However, the NO donors were ineffective in significantly inhibiting the cysteine proteinase activity within the parasites. This finding was confirmed by the ineffectiveness of the NO donors to block proteinolysis in the lysosome of the parasites. The results show that the trypanocidal activity of NO donors cannot be attributed to the inhibition of the major lysosomal cysteine proteinase in bloodstream forms of *T. brucei*.

15099. **Torrie, L. S., Wyllie, S., Spinks, D., Oza, S. L., Thompson, S., Harrison, J. R., Gilbert, I. H., Wyatt, P. G., Fairlamb, A. H. & Frearson, J. A., 2009.** Chemical validation of trypanothione synthetase: a potential drug target for human trypanosomiasis. *Journal of Biological Chemistry*, **284** (52): 36137-36145.

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

In the search for new therapeutics for the treatment of human African trypanosomosis, many potential drug targets in *Trypanosoma brucei* have been validated by genetic means, but very few have been chemically validated. Trypanothione synthetase (TryS; EC 6.3.1.9; spermidine/glutathionylspermidine:glutathione ligase (ADP-forming)) is one such target. To identify novel inhibitors of *T. brucei* TryS, we developed an *in vitro* enzyme assay, which was amenable to high throughput screening. The subsequent screen of a diverse compound library resulted in the identification of three novel series of TryS inhibitors. Further chemical

exploration resulted in leads with nanomolar potency, which displayed mixed, uncompetitive, and allosteric-type inhibition with respect to spermidine, ATP, and glutathione, respectively. Representatives of all three series inhibited growth of bloodstream *T. brucei* *in vitro*. Exposure to one of our lead compounds (DDD86243; 2 x EC50 for 72 h) decreased intracellular trypanothione levels to <10 percent of wild type. In addition, there was a corresponding 5-fold increase in the precursor metabolite, glutathione, providing strong evidence that DDD86243 was acting on target to inhibit TryS. This was confirmed with wild-type, TryS single knock-out, and TryS-overexpressing cell lines showing expected changes in potency to DDD86243. Taken together, these data provide initial chemical validation of TryS as a drug target in *T. brucei*.

15100. **Ulrich, H. & Wrenger, C., 2009.** Disease-specific biomarker discovery by aptamers. *Cytometry A*, **75** (9): 727-733.

Departamento de Bioquímica, Instituto de Química, Universidade de São Paulo, São Paulo, SP, Brazil. [henning@iq.usp.br].

RNA and DNA aptamers developed by an *in vitro* selection process, Systematic Evolution of Ligands by EXponential enrichment (SELEX), comprise a novel class of high-affinity and specific capture agents, which can be easily modified for cytometry and *in vivo* applications. A novel application of this technique (Cell SELEX) explores the expression of cell surface epitopes that differ between two given cell types or between healthy and diseased cells. Using whole cells as targets, aptamer libraries can be identified that bind to biomarkers expressed by target cells and not by any other cells. Aptamers have been developed that specifically interact with cell surface epitopes of trypanosomes or distinguish between the differences in molecular signature of somatic and cancer cells. Aside from its use for target cell identification by image and flow cytometry and laser-scanning microscopy, aptamers can be used for ligand-mediated purification and identification of their binding proteins in target cell membranes. In this review, we discuss an approach for the development of aptamers targeting parasite-derived surface proteins of *Trypanosoma* and *Plasmodium*.

15101. **Yamage, M., Yoshiyama, M., Grab, D. J., Kubo, M., Iwasaki, T., Kitani, H., Ishibashi, J. & Yamakawa, M., 2009.** Characteristics of novel insect defensin-based membrane-disrupting trypanocidal peptides. *Bioscience, Biotechnology and Biochemistry*, **73** (7): 1520-1526.

Innate Immunity Research Unit, National Institute of the Agrobiological Sciences, Tsukuba, Ibaraki, Japan.

Synthetic D- and L-amino acid type cationic 9-mer peptides (all sequences were synthesized as D- or L-amino acids) derived from the active sites of insect defensins were tested for their ability to modify the growth of blood-stream form of African trypanosomes *in vitro*. One of them, the D-type peptide A (RLYLRIGR-NH(2)), irreversibly suppressed proliferation of the *Trypanosoma brucei brucei* GUTat3.1 parasite. The presence of negatively charged phosphatidylserine on the surface of the parasites was demonstrated, suggesting electrostatic interaction between the peptide and the phospholipids. Furthermore, this peptide was found to alter trypanosome membrane-potentials significantly, an effect apparently due to the removal of the parasite's plasma membrane. The potential toxic

effectsof D-peptide A on mammalian cells was assessed using human brain microvascular endothelial cells. Only minor effects were found when the endothelial cells were exposed for 16 h to peptide concentrations of less than 200 µM. These findings suggest that insect defensin-based peptides represent a potentially new class of membrane-disrupting trypanocidal drugs.

15102. **Zohrabi-Kalantari, V., Heidler, P., Kaiser, M., Brun, R., Kamper, C. & Link, A., 2009.** Inhibitors of adenosine consuming parasites through polymer-assisted N-acylation of N(6)-substituted 5' (')-amino-5' (')-deoxyadenosines. *Molecular Diversity*. **e Publication ahead of print, June 26.**

Institute of Pharmaceutical Chemistry, Philipps-University Marburg, Marbacher Weg 6, 35032, Marburg, Germany.

A series of 30 adenosine derivatives with three different substituents at the N(6)-position were prepared in order to evaluate their potential to inhibit the pathogenic protozoa *Plasmodium falciparum* and *Trypanosoma brucei* *in vitro*. The rationale for synthesis of these structures was the high probability of interactions with multiple adenosine associated targets and the assumption that N(6)-substitutents should increase stability against adenosine deaminases and allow the molecules to diffuse across parasite membranes. Starting from inosine, the new compounds were prepared as single isomers using a polymer-assisted acylation protocol enabling the straightforward isolation of the target compounds in pure form. Three of the compounds displayed anti-plasmodial and one anti-trypanosomal activity in the single digit micromolar concentration range.

8. TRYPANOSOME RESEARCH

(a) CULTIVATION OF TRYPANOSOMES

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

15103. **Duffy, C. W., Morrison, L. J., Black, A., Pinchbeck, G. L., Christley, R. M., Schoenefeld, A., Tait, A., Turner, C. M. & MacLeod, A., 2009.** *Trypanosoma vivax* displays a clonal population structure. *International Journal of Parasitology*, **39** (13): 1475-1483.

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

African animal trypanosomosis, or Nagana, is a debilitating and economically costly disease with a major impact on animal health in sub-Saharan Africa. *Trypanosoma vivax*, one of the principal trypanosome species responsible for the disease, infects a wide host range including cattle, goats, horses and donkeys and is transmitted both cyclically by tsetse flies and mechanically by other biting flies, resulting in a distribution covering large swathes of South America and much of sub-Saharan Africa. While there is evidence for mating in some of the related trypanosome species, *Trypanosoma brucei*, *Trypanosoma congolense* and

Trypanosoma cruzi, very little work has been carried out to examine this question in *T. vivax*. Understanding whether mating occurs in *T. vivax* will provide insight into the dynamics of trait inheritance, for example the spread of drug resistance, as well as examining the origins of meiosis in the order Kinetoplastida. With this in mind we have identified orthologues of eight core meiotic genes within the genome, the presence of which imply that the potential for mating exists in this species. In order to address whether mating occurs, we have investigated a sympatric field population of *T. vivax* collected from livestock in The Gambia, using microsatellite markers developed for this species. Our analysis has identified a clonal population structure showing significant linkage disequilibrium, homozygote deficits and disagreement with Hardy-Weinberg predictions at six microsatellite loci, indicative of a lack of mating in this population of *T. vivax*.

15104. **Gibson, W., 2009.** Species-specific probes for the identification of the African tsetse-transmitted trypanosomes. *Parasitology*, **136** (12): 1501-1507.

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

The first step in studying the epidemiology of a disease is the accurate identification of the pathogen. Traditional reliance on morphological identification has given way to the use of molecular methods for the detection and identification of pathogens, greatly improving our understanding of epidemiology. For the African tsetse-transmitted trypanosomes, the growth of PCR methods for identification of trypanosomes has led to increased appreciation of trypanosome genetic diversity and discovery of hitherto unknown trypanosome species, as well as greater knowledge about the number and type of trypanosome infections circulating in mammalian hosts and vectors. Sequence data and phylogenetic analysis have provided quantitative information on the relatedness of different trypanosome species and allowed the new trypanosome genotypes discovered through the use of species identification methods in the field to be accurately placed in the phylogenetic tree.

15105. **Lai, D. H., Wang, Q. P., Li, Z., Luckins, A. G., Reid, S. A. & Lun, Z. R., 2009.** Investigations into human serum sensitivity expressed by stocks of *Trypanosoma brucei evansi*. *International Journal of Parasitology*. **e-publication ahead of print, December 5.**

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

Trypanosoma brucei evansi, a widely distributed species of trypanosome infecting different livestock species in many countries in Africa, Asia and South America, has recently been reported as a pathogen causing a case of human trypanosomosis in India. To date, there is little information regarding the natural resistance of animal-infective stocks of *T. b. evansi* to normal human serum (NHS). In this study, we investigated the degree of sensitivity to NHS of 15 stocks of *T. b. evansi* from different geographical origins and found that 10 of the stocks were completely susceptible to the action of NHS; parasites disappeared from the blood of infected mice within a few hours and the mice remained free from infection for more

than one month. The remaining five stocks were partially resistant to NHS; although parasites initially disappeared from the circulation more than 50 percent of the mice showed relapse infection 10-18d later. Studies on one stock, *T. b. evansi* STIB 810, showed that the changes in parasitaemia in the infected mice were correlated with the amount of NHS inoculated (correlation factor -0.584 and $P=0.001$). When this stock was passaged 25 times in mice in the presence of NHS it was found that the trypanosomes' serum resistance increased compared with the parent stock from which they were derived; 40 percent of the passaged parasites survived after *in vitro* incubation with 50 percent NHS for 7h, while only 1 percent of individual trypanosomes of the parent stock survived under the same conditions. These findings show for the first time that human serum sensitivity varies amongst stocks of *T. b. evansi*, that some of them naturally display resistance to NHS and that, furthermore, *T. b. evansi* serum resistance can be increased by sub-passage in the presence of NHS.

15106. **Sykes, M. L. & Avery, V. M., 2009.** A luciferase based viability assay for ATP detection in 384-well format for high throughput whole cell screening of *Trypanosoma brucei brucei* bloodstream form strain 427. *Parasite Vectors*, **2** (1): 54.

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Eskitis Building N27, Brisbane Innovation Park, Don Young Road, Nathan, Queensland, Australia. [v.avery@griffith.edu.au].

Human African Trypanosomosis (HAT) is caused by two trypanosome species, *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Current drugs available for the treatment of HAT have significant issues related to toxicity, administration regimes with limited effectiveness across species and disease stages, thus there is a considerable need to find alternative drugs. A well recognized approach to identify new drug candidates is high throughput screening (HTS) of large compound library collections. We describe here the development of a luciferase based viability assay in 384-well plate format suitable for HTS of *T. b. brucei*. The parameters that were explored to determine the final HTS assay conditions are described in detail and include DMSO tolerability, Z', diluents and cell inoculum density. Reference compound activities were determined for diminazene, staurosporine and pentamidine and compared with previously published IC50 data obtained. The assay has a comparable sensitivity to reference drugs and is more cost effective than the 96-well format currently reported for *T. b. brucei*. Due to the reproducibility and sensitivity of this assay it is recommended for potential HTS application. As it is commercially available this assay can also be utilized in many laboratories for both large and small scale screening.

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

15107. **Acestor, N., Panigrahi, A. K., Ogata, Y., Anupama, A. & Stuart, K. D., 2009.** Protein composition of *Trypanosoma brucei* mitochondrial membranes. *Proteomics*, **9** (24): 5497-5508.

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

Mitochondria consist of four compartments, outer membrane, intermembrane space, inner membrane, and matrix; each harbouring specific functions and structures. In this study,

we used LC-MS/MS to characterize the protein composition of *Trypanosoma brucei* mitochondrial (mt) membranes, which were enriched by different biochemical fractionation techniques. The analyses identified 202 proteins that contain one or more transmembrane domain(s) and/or positive GRAVY scores. Of these, various criteria were used to assign 72 proteins to mt membranes with high confidence, and 106 with moderate-to-low confidence. The sub-cellular localization of a selected subset of 13 membrane assigned proteins was confirmed by tagging and immunofluorescence analysis. While most proteins assigned to mt membrane have putative roles in metabolic, energy generating, and transport processes, approximately 50 percent have no known function. These studies result in a comprehensive profile of the composition and sub-organellar location of proteins in the *T. brucei* mitochondrion thus, providing useful information on mt functions.

15108. **Aeby, E., Seidel, V. & Schneider, A., 2009.** The selenoproteome is dispensable in bloodstream forms of *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **168** (2): 191-193.

Department of Chemistry and Biochemistry, University of Bern, Freiestr. 3, CH-3012 Bern, Switzerland.

Here we show that absence of Sep-tRNA:Sec-tRNA synthase (SepSecS) a key enzyme required for the synthesis of the three trypanosomal selenoproteins does not affect growth of bloodstream forms of *Trypanosoma brucei*. Both life cycle stages of *T. brucei* are highly sensitive to auranofin, a compound known to target selenoproteins. However, the same sensitivity is observed in the SepSecS double knockout cell lines indicating that the trypanocidal action of auranofin is not connected to selenoproteins. Finally, we show that absence of selenoproteins does not increase sensitivity to H₂O₂-induced oxidative stress. Thus in cell culture normal growth of procyclic and bloodstream *T. brucei* does not depend on selenoproteins.

15109. **Alsford, S., Horn, D. & Glover, L., 2009.** DNA breaks as triggers for antigenic variation in African trypanosomes. *Genome Biology*, **10** (6): 223.

London School of Hygiene & Tropical Medicine, Keppel Street, London WC1E7HT, UK.

The DNA repair machinery has been co-opted for antigenic variation in African trypanosomes. New work directly demonstrates that a double-strand break initiates a switch in the expressed variant surface coat.

15110. **Amlabu, E., Nok, A. J. & Sallau, A. B., 2009.** Purification and biochemical characterization of lysosomal acid phosphatases (EC 3.1.3.2) from blood stream forms, *Trypanosoma brucei brucei*. *Parasitology International*, **58** (3): 238-242.

Department of Biochemistry, Ahmadu Bello University, Zaria, Nigeria. [ninmac2000@yahoo.com].

15111. **Aphasizheva, I., Ringpis, G. E., Weng, J., Gershon, P. D., Lathrop, R. H. & Aphasizhev, R., 2009.** Novel TUTase associates with an editosome-like complex

in mitochondria of *Trypanosoma brucei*. *Rna*, **15** (7): 1322-1337.

Department of Microbiology and Molecular Genetics, School of Medicine,
University of California at Irvine, Irvine, California 92697, USA.

15112. **Archer, S. K., Luu, V. D., de Queiroz, R. A., Brems, S. & Clayton, C., 2009.**
Trypanosoma brucei PUF9 regulates mRNAs for proteins involved in replicative
processes over the cell cycle. *PLoS Pathogens*, **5** (8): e1000565.

Zentrum für Molekulare Biologie Heidelberg, DKFZ-ZMBH Allianz,
Heidelberg, Germany. [s.archer@zmbh.uni-heidelberg.de].

Many genes that are required at specific points in the cell cycle exhibit cell cycle-dependent expression. In the early-diverging model eukaryote and important human pathogen *Trypanosoma brucei*, regulation of gene expression in the cell cycle and other processes is almost entirely post-transcriptional. Here, we show that the *T. brucei* RNA-binding protein PUF9 stabilizes certain transcripts during S-phase. Target transcripts of PUF9--LIGKA, PNT1 and PNT2--were identified by affinity purification with TAP-tagged PUF9. RNAi against PUF9 caused an accumulation of cells in G2/M phase and unexpectedly destabilized the PUF9 target mRNAs, despite the fact that most known Puf-domain proteins promote degradation of their target mRNAs. The levels of the PUF9-regulated transcripts were cell cycle dependent, peaking in mid- to late- S-phase, and this effect was abolished when PUF9 was targeted by RNAi. The sequence UUGUACC was over-represented in the 3' UTRs of PUF9 targets; a point mutation in this motif abolished PUF9-dependent stabilization of a reporter transcript carrying the PNT1 3' UTR. LIGKA is involved in replication of the kinetoplast, and here we show that PNT1 is also kinetoplast-associated and its over-expression causes kinetoplast-related defects, while PNT2 is localized to the nucleus in G1 phase and redistributes to the mitotic spindle during mitosis. PUF9 targets may constitute a post-transcriptional regulon, encoding proteins involved in temporally coordinated replicative processes in early G2 phase.

15113. **Bachega, J. F., Navarro, M. V., Bleicher, L., Bortoleto-Bugs, R. K., Dive, D., Hoffmann, P., Viscogliosi, E. & Garratt, R. C., 2009.** Systematic structural studies of iron superoxide dismutases from human parasites and a statistical coupling analysis of metal binding specificity. *Proteins*, **77** (1): 26-37.

Institute of Physics of Sao Carlos, University of Sao Paulo, CEP 13560-970,
Sao Carlos, SP, Brazil.

Superoxide dismutases (SODs) are a crucial class of enzymes in the combat against intracellular free radical damage. They eliminate superoxide radicals by converting them into hydrogen peroxide and oxygen. In spite of their very different life cycles and infection strategies, the human parasites *Plasmodium falciparum*, *Trypanosoma cruzi* and *Trypanosoma brucei* are known to be sensitive to oxidative stress. Thus the parasite Fe-SODs have become attractive targets for novel drug development. Here we report the crystal structures of FeSODs from the trypanosomes *T. brucei* at 2.0 Å and *T. cruzi* at 1.9 Å resolution, and that from *P. falciparum* at a higher resolution (2.0 Å) to that previously reported. The homodimeric enzymes are compared with the related human MnSOD with

particular attention to structural aspects which are relevant for drug design. Although the structures possess a very similar overall fold, differences between the enzymes at the entrance to the channel which leads to the active site could be identified. These lead to a slightly broader and more positively charged cavity in the parasite enzymes. Furthermore, a statistical coupling analysis (SCA) for the whole Fe/MnSOD family reveals different patterns of residue coupling for Mn and Fe SODs, as well as for the dimeric and tetrameric states. In both cases, the statistically coupled residues lie adjacent to the conserved core surrounding the metal center and may be expected to be responsible for its fine tuning, leading to metal ion specificity.

15114. **Bahia, D., Oliveira, L. M., Lima, F. M., Oliveira, P., Silveira, J. F., Mortara, R. A. & Ruiz, J. C., 2009.** The TryPIKinome of five human pathogenic trypanosomatids: *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, *Leishmania braziliensis* and *Leishmania infantum*-new tools for designing specific inhibitors. *Biochemical and Biophysical Research Communications*, **390** (3): 963-970.

Escola Paulista de Medicina, UNIFESP, Sao Paulo, Brazil.
[dianabahia@hotmail.com].

Phosphatidylinositol (PI) kinases are at the heart of one of the major pathways of intracellular signal transduction. Here, we present the first report on a survey made by similarity searches against the five human pathogenic trypanosomatids *Trypanosoma brucei*, *Trypanosoma cruzi*, *Leishmania major*, *Leishmania braziliensis* and *Leishmania infantum* genomes available to date for phosphatidylinositol- and related-kinases (TryPIKs). In addition to generating a panel called "The TryPIKinome", we propose a model of signaling pathways for these TryPIKs. The involvement of TryPIKs in fundamental pathways, such as intracellular signal transduction and host invasion processes, makes the study of TryPIKs an important area for further inquiry. New subtype-specific inhibitors are expected to work on individual members of the PIK family and, therefore, can presumably neutralize trypanosomatid invasion processes.

15115. **Banerjee, H., Palenchar, J. B., Lukaszewicz, M., Bojarska, E., Stepinski, J., Jemielity, J., Guranowski, A., Ng, S., Wah, D. A., Darzynkiewicz, E. & Bellofatto, V., 2009.** Identification of the HIT-45 protein from *Trypanosoma brucei* as an FHIT protein/dinucleoside triphosphatase: substrate specificity studies on the recombinant and endogenous proteins. *Rna*, **15** (8): 1554-1564.

Department of Microbiology and Molecular Genetics, University of Medicine and Dentistry-New Jersey Medical School, Newark, New Jersey 07103, USA.

15116. **Bayele, H. K., 2009.** *Trypanosoma brucei*: a putative RNA polymerase II promoter. *Experimental Parasitology*, **123** (4): 313-318.

Department of Structural & Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK. [bayele@biochem.ucl.ac.uk].

RNA polymerase II (pol II) promoters are rare in the African trypanosome *Trypanosoma brucei* because gene regulation in the parasite is complex and polycistronic. Here, we describe a putative pol II promoter and its structure-function relationship. The promoter has features of an archetypal eukaryotic pol II promoter including putative canonical CCAAT and TATA boxes, and an initiator element. However, the spatial arrangement of these elements is only similar to yeast pol II promoters. Deletion mapping and transcription assays enabled delineation of a minimal promoter that could drive orientation-independent reporter gene expression suggesting that it may be a bidirectional promoter. *In vitro* transcription in a heterologous nuclear extract revealed that the promoter can be recognized by the basal eukaryotic transcription complex. This suggests that the transcription machinery in the parasite may be very similar to those of other eukaryotes.

15117. **Bercovich, N., Levin, M. J., Clayton, C. & Vazquez, M. P., 2009.** Identification of core components of the exon junction complex in trypanosomes. *Molecular and Biochemical Parasitology*, **166** (2): 190-193.

INGEBI-CONICET, Vuelta de Obligado 2490, 2P, Buenos Aires, Argentina.

In animal cells, the exon junction complex (EJC) is deposited onto mRNAs during the second step of splicing, 20-24 nt upstream of the exon-exon junction. The EJC core contains four proteins: Mago, Y14, eIF4AIII and Btz. In trypanosomes, cis-splicing is very rare but all mRNAs are subject to 5'trans-splicing of a 39-nt RNA sequence. Here we show that trypanosomes have a conserved Mago and a divergent Y14 protein, but we were unable to identify a Btz orthologue. We demonstrate that Mago and Y14 form a stable heterodimer using yeast two hybrid analyses. We also show that this complex co-purifies *in vivo* in trypanosomes with a protein containing an NTF2 domain, typically involved in mRNA transport.

15118. **Bocedi, A., Dawood, K. F., Fabrini, R., Federici, G., Gradoni, L., Pedersen, J. Z. & Ricci, G., 2009.** Trypanothione efficiently intercepts nitric oxide as a harmless iron complex in trypanosomatid parasites. *The FASEB Journal*. **Published online before print December 1.**

Department of Biology, University of Rome Roma Tre, Rome, Italy; Department of Chemical Sciences and Technologies and Department of Biology, University of Rome Tor Vergata, Rome, Italy; Children's Hospital Istituto di Ricovero e Cura a Carattere Scientifico Bambin Gesù, Rome, Italy; and Department of Infectious, Parasitic, and Immunomediated Diseases, Istituto Superiore di Sanita, Rome, Italy. [riccig@uniroma2.it].

Trypanosomatids are protozoan organisms that cause serious diseases, including African sleeping sickness, Chagas disease, and leishmaniasis, affecting about 30 million people in the world. These parasites contain the unusual dithiol trypanothione [T(SH)₂] instead of glutathione (GSH) as the main intracellular reductant, and they have replaced the otherwise ubiquitous GSH/glutathione reductase redox couple with a T(SH)₂/trypanothione reductase (TR) system. The reason for the existence of T(SH)₂ in parasitic organisms has remained an enigma. Here, we show that T(SH)₂ is able to intercept nitric oxide and labile iron and form a dinitrosyl-iron complex with at least 600 times higher affinity than GSH.

Accumulation of the paramagnetic dinitrosyl-trypanothionyl iron complex *in vivo* was observed in *Trypanosoma brucei* and *Leishmania infantum* exposed to nitric oxide. While the analogous dinitrosyl-diglutathionyl iron complex formed in mammalian cells is a potent irreversible inhibitor of glutathione reductase (IC₅₀=4 μM), the T(SH)₂ complex does not inactivate TR even at millimolar levels. The peculiar capacity of T(SH)₂ to sequester NO and iron in a harmless stable complex could explain the predominance of this thiol in parasites regularly exposed to NO.

15119. **Bringaud, F., Berriman, M. & Hertz-Fowler, C., 2009.** Trypanosomatid genomes contain several subfamilies of ingi-related retroposons. *Eukaryotic Cell*, **8** (10): 1532-1542.

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

Retroposons are ubiquitous transposable elements found in the genomes of most eukaryotes, including trypanosomatids. The African and American trypanosomes (*Trypanosoma brucei* and *Trypanosoma cruzi*) contain long autonomous retroposons of the ingi clade (Tbingi and L1Tc, respectively) and short nonautonomous truncated versions (TbRIME and NARTc, respectively), as well as degenerate ingi-related retroposons devoid of coding capacity (DIREs). In contrast, *Leishmania major* contains only remnants of extinct retroposons (*LmDIREs*) and of short nonautonomous heterogeneous elements (*LmSIDERs*). We extend this comparative and evolutionary analysis of retroposons to the genomes of two other African trypanosomes (*Trypanosoma congolense* and *Trypanosoma vivax*) and another *Leishmania* sp. (*Leishmania braziliensis*). Three new potentially functional retroposons of the ingi clade have been identified: Tvingi in *T. vivax* and Tcoingi and L1Tco in *T. congolense*. *T. congolense* is the first trypanosomatid containing two classes of potentially active retroposons of the ingi clade. We analysed sequences located upstream of these new long autonomous ingi-related elements, which code for the recognition site of the retroposon-encoded endonuclease. The closely related Tcoingi and Tvingi elements show the same conserved pattern, indicating that the Tcoingi- and Tvingi-encoded endonucleases share site specificity. Similarly, the conserved pattern previously identified upstream of L1Tc has also been detected at the same relative position upstream of L1Tco elements. A phylogenetic analysis of all ingi-related retroposons identified so far, including DIREs, clearly shows that several distinct subfamilies have emerged and coexisted, though in the course of trypanosomatid evolution, only a few have been maintained as active elements in modern trypanosomatid (sub)species.

15120. **Bruske, E. I., Sendfeld, F. & Schneider, A., 2009.** Thiolated tRNAs of *Trypanosoma brucei* are imported into mitochondria and dethiolated after import. *Journal of Biological Chemistry*, **284** (52): 36491-36499.

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, CH-3012 Bern, Switzerland.

All mitochondrial tRNAs in *Trypanosoma brucei* derive from cytosolic tRNAs that are in part imported into mitochondria. Some trypanosomal tRNAs are thiolated in a

compartment-specific manner. We have identified three proteins required for the thio modification of cytosolic tRNA(Gln), tRNA(Glu), and tRNA(Lys). RNA interference-mediated ablation of these proteins results in the cytosolic accumulation non-thio-modified tRNAs but does not increase their import. Moreover, *in vitro* import experiments showed that both thio-modified and non-thio-modified tRNA(Glu) can efficiently be imported into mitochondria. These results indicate that unlike previously suggested, the cytosol-specific thio modifications do not function as antideterminants for mitochondrial tRNA import. Consistent with these results we showed by using inducible expression of a tagged tRNA(Glu) that it is mainly the thiolated form that is imported *in vivo*. Unexpectedly, the imported tRNA becomes dethiolated after import, which explains why the non-thiolated form is enriched in mitochondria. Finally, we have identified two genes required for thiolation of imported tRNA(Trp) whose wobble nucleotide is subject to mitochondrial C to U editing. Interestingly, down-regulation of thiolation resulted in an increase of edited tRNA(Trp) but did not affect growth.

15121. **Chamond, N., Cosson, A., Coatnoan, N. & Minoprio, P., 2009.** Proline racemases are conserved mitogens: characterization of a *Trypanosoma vivax* proline racemase. *Molecular and Biochemical Parasitology*, **165** (2): 170-179.

Institut Pasteur, Laboratoire d'Immunobiologie des Infections a *Trypanosoma*,
Département d'Immunologie, Paris, France.

Trypanosoma cruzi proline racemases (TcPRAC) are the only eukaryotic proline racemases described so far. Except their role in the interconversion of free L- and D-proline enantiomers, parasite TcPRACs are involved in major *T. cruzi* biological pathways. These essential enzymes are implicated in the process of parasite differentiation and the acquisition of virulence during metacyclogenesis and are currently considered as key targets for drug development against Chagas' disease. In this study, we searched for the presence of TcPRAC gene homologues among other trypanosomatid genomes. Despite the high degree of gene synteny observed in Kinetoplastidae genomes, PRAC genes are missing in *Trypanosoma brucei*, *Trypanosoma congolense* and *Leishmania* spp. genomes. Interestingly, we identified a hypothetical PRAC gene in *Trypanosoma vivax* that is the major haemoparasite responsible for livestock trypanosomosis, a serious economical impact for most of African and South American countries. We report here that the product of this *T. vivax* gene is *bona fide* a proline racemase with an activity comparable to the one we described previously for TcPRAC. Inhibition studies using the pyrrole-2-carboxylic acid confirmed that this compound is a competitive inhibitor for both TcPRAC and TvPRAC enzymes. Similarly to TcPRAC and all members of the racemase family studied so far in other pathogenic and nosocomial bacteria, our results show that TvPRAC is a T-cell-independent B-cell mitogen. Therefore the product of the novel TvPRAC gene identified in *T. vivax* and reported here has the potential to be used as a drug target for this parasite-based disease.

15122. **Clemmens, C. S., Morris, M. T., Lyda, T. A., Acosta-Serrano, A. & Morris, J. C., 2009.** *Trypanosoma brucei* AMP-activated kinase subunit homologues influence surface molecule expression. *Experimental Parasitology*, **123** (3): 250-257.

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

The African trypanosome, *Trypanosoma brucei*, can gauge its environment by sensing nutrient availability. For example, procyclic form (PF) trypanosomes monitor changes in glucose levels to regulate surface molecule expression, which is important for survival in the tsetse fly vector. The molecular connection between glycolysis and surface molecule expression is unknown. Here we partially characterize *T. brucei* homologues of the beta and gamma subunits of the AMP-activated protein kinase (AMPK), and determine their roles in regulating surface molecule expression. Using flow cytometry and mass spectrometry, we found that TbAMPKbeta or TbAMPKgamma-deficient parasites express both of the major surface molecules, EP- and GPEET-procyclicin, with the latter being a form that is expressed when glucose is low such as in the tsetse fly. Last, we have found that the putative scaffold component of the complex, TbAMPKbeta, fractionates with organellar components and colocalizes in part with a glycosomal marker as well as the flagellum of PF parasites.

15123. **Colasante, C., Pena Diaz, P., Clayton, C. & Voncken, F., 2009.** Mitochondrial carrier family inventory of *Trypanosoma brucei brucei*: identification, expression and subcellular localisation. *Molecular and Biochemical Parasitology*, **167** (2): 104-117.

Department of Biological Sciences and Hull York Medical School (HYMS),
University of Hull, HU6 7RX Hull, UK. [colasanteclaudia@gmx.de].

The mitochondrial carrier family (MCF) is a group of structurally conserved proteins that mediate the transport of a wide range of metabolic intermediates across the mitochondrial inner membrane. In this paper, an overview of the mitochondrial carrier proteins (MCPs) of the early-branching kinetoplastid parasite *Trypanosoma brucei brucei* is presented. Sequence analysis and phylogenetic reconstruction gave insight into the evolution and conservation of the 24 identified TbMCPs; for most of these, putative transport functions could be predicted. Comparison of the kinetoplastid MCP inventory to those previously reported for other eukaryotes revealed remarkable deviations: *T. b. brucei* lacks genes encoding some prototypical MCF members, such as the citrate carrier and uncoupling proteins. The *in vivo* expression of the identified TbMCPs in the two replicating life-cycle forms of *T. b. brucei*, the bloodstream-form and procyclic-form, was quantitatively assessed at the mRNA level by Northern blot analysis. Immunolocalisation studies confirmed that majority of the 24 identified TbMCPs is found in the mitochondrion of procyclic-form *T. b. brucei*.

15124. **Das, A. & Bellofatto, V., 2009.** The non-canonical CTD of RNAP-II is essential for productive RNA synthesis in *Trypanosoma brucei*. *PLoS One*, **4** (9): e6959.

Department of Microbiology and Molecular Genetics, University of Medicine
and Dentistry-New Jersey Medical School, Newark, New Jersey, USA. [A.Das-
dasak@umdnj.edu].

The carboxy-terminal domain (CTD) of the largest subunit (RPB1) of RNA polymerase II (RNAP-II) is essential for gene expression in metazoa and yeast. The canonical CTD is characterized by heptapeptide repeats. Differential phosphorylation of canonical CTD orchestrates transcriptional and co-transcriptional maturation of mRNA and snRNA. Many

organisms, including trypanosomes, lack a canonical CTD. In these organisms, the CTD is called a non-canonical CTD or pseudo-CTD (PsiCTD). In the African trypanosome, *Trypanosoma brucei*, the PsiCTD is approximately 285 amino acids long, rich in serines and prolines, and phosphorylated. We report that *T. brucei* RNAP-II lacking the entire PsiCTD or containing only a 95-amino-acid-long PsiCTD failed to support cell viability. In contrast, RNAP-II with a 186-amino-acid-long PsiCTD maintained cellular growth. RNAP-II with PsiCTD truncations resulted in abortive initiation of transcription. These data establish that non-canonical CTDs play an important role in gene expression.

15125. **Dean, S., Marchetti, R., Kirk, K. & Matthews, K. R., 2009.** A surface transporter family conveys the trypanosome differentiation signal. *Nature*, **459** (7244): 213-217.

Centre for Immunity, Infection and Evolution, Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, West Mains Road, Edinburgh EH9 3JT, UK.

Microbial pathogens use environmental cues to trigger the developmental events needed to infect mammalian hosts or transmit to disease vectors. The parasites causing African sleeping sickness respond to citrate or cis-aconitate (CCA) to initiate life-cycle development when transmitted to their tsetse fly vector. This requires hypersensitization of the parasites to CCA by exposure to low temperature, conditions encountered after tsetse fly feeding at dusk or dawn. Here we identify a carboxylate-transporter family, PAD (proteins associated with differentiation), required for perception of this differentiation signal. Consistent with predictions for the response of trypanosomes to CCA, PAD proteins are expressed on the surface of the transmission-competent “stumpy-form” parasites in the bloodstream, and at least one member is thermoregulated, showing elevated expression and surface access at low temperature. Moreover, RNA-interference-mediated ablation of PAD expression diminishes CCA-induced differentiation and eliminates CCA hypersensitivity under cold-shock conditions. As well as being molecular transducers of the differentiation signal in these parasites, PAD proteins provide the first example of a surface marker able to discriminate the transmission stage of trypanosomes in their mammalian host.

15126. **DeGrasse, J. A., DuBois, K. N., Devos, D., Siegel, T. N., Sali, A., Field, M. C., Rout, M. P. & Chait, B. T., 2009.** Evidence for a shared nuclear pore complex architecture that is conserved from the last common eukaryotic ancestor. *Molecular and Cellular Proteomics*, **8** (9): 2119-2130.

Laboratory of Mass Spectrometry and Gaseous Ion Chemistry, The Rockefeller University, New York, New York 10065, USA.

The nuclear pore complex (NPC) is a macromolecular assembly embedded within the nuclear envelope that mediates bidirectional exchange of material between the nucleus and cytoplasm. Our recent work on the yeast NPC has revealed a simple modularity in its architecture and suggested a common evolutionary origin of the NPC and vesicle coating complexes in a progenitor protocoatome. However, detailed compositional and structural information is currently only available for vertebrate and yeast NPCs, which are evolutionarily closely related. Hence our understanding of NPC composition in a full

evolutionary context is sparse. Moreover despite the ubiquitous nature of the NPC, sequence searches in distant taxa have identified surprisingly few NPC components, suggesting that much of the NPC may not be conserved. Thus, to gain a broad perspective on the origins and evolution of the NPC, we performed proteomics analyses of NPC-containing fractions from a divergent eukaryote (*Trypanosoma brucei*) and obtained a comprehensive inventory of its nucleoporins. Strikingly trypanosome nucleoporins clearly share with metazoa and yeast their fold type, domain organization, composition, and modularity. Overall these data provide conclusive evidence that the majority of NPC architecture is indeed conserved throughout the Eukaryota and was already established in the last common eukaryotic ancestor. These findings strongly support the hypothesis that NPCs share a common ancestry with vesicle coating complexes and that both were established very early in eukaryotic evolution.

15127. **Demonchy, R., Blisnick, T., Deprez, C., Toutirais, G., Loussert, C., Marande, W., Grellier, P., Bastin, P. & Kohl, L., 2009.** Kinesin 9 family members perform separate functions in the trypanosome flagellum. *Journal of Cell Biology*, **187** (5): 615-622.

Adaptation Processes of Protozoa to their Environment, Centre National de la Recherche Scientifique FRE3206, 75231 Paris, France.

15128. **Espanol, Y., Thut, D., Schneider, A. & de Poupplana, L. R., 2009.** A mechanism for functional segregation of mitochondrial and cytosolic genetic codes. *Proceedings of the National Academy of Sciences U S A*, **106** (46): 19420-19425.

Institute for Research in Biomedicine, Barcelona, Catalonia, Spain.

The coexistence of multiple gene translation machineries is a feature of eukaryotic cells and a result of the endosymbiotic events that gave rise to mitochondria, plastids, and other organelles. The conditions required for the integration of these apparatuses within a single cell are not understood, but current evidence indicates that complete ablation of the mitochondrial protein synthesis apparatus and its substitution by its cytosolic equivalent is not possible. Why certain mitochondrial components and not others can be substituted by cytosolic equivalents is not known. In trypanosomatids this situation reaches a limit, because certain aminoacyl-tRNA synthetases are mitochondrial specific despite the fact that all tRNAs in these organisms are shared between cytosol and mitochondria. Here we report that a mitochondria-specific lysyl-tRNA synthetase in *Trypanosoma* has evolved a mechanism to block the activity of the enzyme during its synthesis and translocation. Only when the enzyme reaches the mitochondria is it activated through the cleavage of a C-terminal structural extension, preventing the possibility of the enzyme being active in the cytosol.

15129. **Field, M. C. & Carrington, M., 2009.** The trypanosome flagellar pocket. *Nature Reviews Microbiology*, **7** (11): 775-786.

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

Trypanosomes are important disease agents and excellent models for the study of evolutionary cell biology. The trypanosome flagellar pocket is a small invagination of the

plasma membrane where the flagellum exits the cytoplasm and participates in many cellular processes. It is the only site of exocytosis and endocytosis and part of a multiorganelle complex that is involved in cell polarity and cell division. Several flagellar pocket-associated proteins have been identified and found to contribute to trafficking and virulence. In this review we discuss the contribution of the flagellar pocket to protein trafficking, immune evasion and other processes.

15130. **Figueiredo, L. M., Cross, G. A. & Janzen, C. J., 2009.** Epigenetic regulation in African trypanosomes: a new kid on the block. *Nature Reviews Microbiology*, 7 (7): 504-513.

Laboratory of Molecular Parasitology, The Rockefeller University, 1230 York Avenue, New York, New York 10065, USA.
[luisa.figueiredo@rockefeller.edu].

Epigenetic regulation is important in many facets of eukaryotic biology. Recent work has suggested that the basic mechanisms underlying epigenetic regulation extend to eukaryotic parasites. The identification of post-translational histone modifications and chromatin-modifying enzymes is beginning to reveal both common and novel functions for chromatin in these parasites. In this review, we compare the role of epigenetics in African trypanosomes and humans in several biological processes. We discuss how the study of trypanosome chromatin might help us to better understand the evolution of epigenetic processes.

15131. **Fragoso, C. M., Schumann Burkard, G., Oberle, M., Renggli, C. K., Hilzinger, K. & Roditi, I., 2009.** PSSA-2, a membrane-spanning phosphoprotein of *Trypanosoma brucei* is required for efficient maturation of infection. *PLoS One*, 4 (9): e7074.

Institut für Zellbiologie, Universität Bern, Bern, Switzerland.

The coat of *Trypanosoma brucei* consists mainly of glycosylphosphatidylinositol-anchored proteins that are present in several million copies and are characteristic of defined stages of the life cycle. While these major components of the coats of bloodstream forms and procyclic (insect midgut) forms are well characterized, very little is known about less abundant stage-regulated surface proteins and their roles in infection and transmission. By creating epitope-tagged versions of procyclic-specific surface antigen 2 (PSSA-2) we demonstrated that it is a membrane-spanning protein that is expressed by several different life cycle stages in tsetse flies, but not by parasites in the mammalian bloodstream. In common with other membrane-spanning proteins in *T. brucei*, PSSA-2 requires its cytoplasmic domain in order to exit the endoplasmic reticulum. Correct localization of PSSA-2 requires phosphorylation of a cytoplasmic threonine residue (T(305)), a modification that depends on the presence of TbMAPK4. Mutation of T(305) to alanine (T(305)A) has no effect on the localization of the protein in cells that express wild type PSSA-2. In contrast, this protein is largely intracellular when expressed in a null mutant background. A variant with a T(305)D mutation gives strong surface expression in both the wild type and null mutant, but slows growth of the cells, suggesting that it may function as a dominant negative mutant. The PSSA-2 null mutant exhibits no perceptible phenotype in culture and is fully competent at

establishing midgut infections in tsetse, but is defective in colonising the salivary glands and the production of infectious metacyclic forms. Given the protein's structure and the effects of mutation of T(305) on proliferation and localisation, we postulate that PSSA-2 might sense and transmit signals that contribute to the parasite's decision to divide, differentiate or migrate.

15132. **Gadelha, C., Rothery, S., Morphew, M., McIntosh, J. R., Severs, N. J. & Gull, K., 2009.** Membrane domains and flagellar pocket boundaries are influenced by the cytoskeleton in African trypanosomes. *Proceedings of the National Academy of Sciences U S A*, **106** (41): 17425-17430.

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

A key feature of immune evasion for African trypanosomes is the functional specialisation of their surface membrane in an invagination known as the flagellar pocket (FP), the cell's sole site of endocytosis and exocytosis. The FP membrane is biochemically distinct yet continuous with those of the cell body and the flagellum. The structural features maintaining this individuality are not known, and we lack a clear understanding of how extracellular components gain access to the FP. Here, we have defined domains and boundaries on these surface membranes and identified their association with internal cytoskeletal features. The FP membrane appears largely homogeneous and uniformly involved in endocytosis. However, when endocytosis is blocked, receptor-mediated and fluid-phase endocytic markers accumulate specifically on membrane associated with four specialized microtubules in the FP region. These microtubules traverse a distinct boundary and associate with a channel that connects the FP lumen to the extracellular space, suggesting that the channel is the major transport route into the FP.

15133. **Glover, L. & Horn, D., 2009.** Site-specific DNA double-strand breaks greatly increase stable transformation efficiency in *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **166** (2): 194-197.

London School of Hygiene & Tropical Medicine, Keppel Street, London, WC1E 7HT, UK.

Genetic manipulation in African trypanosomes typically relies upon electroporation with chromosomal integration of DNA constructs by homologous recombination. Relatively little is known about chromosomal recombination and repair in these organisms however, and low transformation efficiency and position effects can limit forward genetic approaches. In yeast and mammalian cells, site-specific DNA double-strand breaks (DSBs) stimulate targeted integration through homologous recombination-based repair where the exogenous DNA serves as the template. We have explored the effect of DSBs on targeted integration in bloodstream-form *Trypanosoma brucei*, focusing on the ribosomal RNA-spacer target commonly used to integrate recombinant constructs. DSB-repair within the ribosomal RNA tandem gene-repeats is likely dominated by single-strand annealing allowing approximately 80 percent of cells to survive the break. In the presence of exogenous DNA, transformation efficiency is increased approximately 250-fold by DSB-induction. In the example presented,

more than 1 percent of cells that survive the procedure were transformed generating 80 000 transformants from a typical experiment.

15134. **Godoy, P. D., Nogueira-Junior, L. A., Paes, L. S., Cornejo, A., Martins, R. M., Silber, A. M., Schenkman, S. & Elias, M. C., 2009.** Trypanosome prereplication machinery contains a single functional *orc1/cdc6* protein, which is typical of archaea. *Eukaryotic Cell*, **8** (10): 1592-1603.

Laboratorio de Parasitologia, Instituto Butantan, Av. Vital Brasil, 1500, Sao Paulo 05503900, Brazil.

15135. **Gourguechon, S. & Wang, C. C., 2009.** CRK9 contributes to regulation of mitosis and cytokinesis in the procyclic form of *Trypanosoma brucei*. *BMC Cell Biology*, **10**: 68.

Department of Pharmaceutical Chemistry, University of California, San Francisco, CA 94158-2280, USA. [stephane.gourguechon@berkeley.edu].

The *Trypanosoma brucei* cell cycle is regulated by combinations of cyclin/CRKs (*cdc2* related kinases). Recently, two additional cyclins (*CYC10*, *CYC11*) and six new CRK (*CRK7-12*) homologues were identified in the *T. brucei* genome database 12. Individual RNAi knockdowns of these new proteins in the procyclic form of *T. brucei* showed no apparent phenotype except for the CRK9 depletion, which enriched the cells in G2/M phase. But a similar CRK9 knockdown in the bloodstream form caused no apparent phenotype. CRK9 lacks the typical PSTAIRE motif for cyclin binding and the phenylalanine "gatekeeper" but binds to cyclin B2 *in vitro* and localizes to the nucleus in both forms of *T. brucei*. CRK9-depleted procyclic-form generated no detectable anucleate cells, suggesting an inhibition of cytokinesis by CRK9 depletion as well. The knockdown enriched cells with one nucleus, one kinetoplast and two closely associated basal bodies with an average distance of 1.08 μm in between, which was shorter than the control value of 1.36 μm , and the cells became morphologically deformed and rounded with time. It is concluded that CRK9 may play a role in mediating the segregation between the two kinetoplast/basal body pairs prior to cytokinetic initiation. Since such segregation over a relatively significant distance is essential for cytokinetic initiation only in the procyclic but may not be in the bloodstream form, CRK9 could be specifically involved in regulating cytokinetic initiation in the procyclic form of *T. brucei*.

15136. **Grab, D. J., Garcia-Garcia, J. C., Nikolskaia, O. V., Kim, Y. V., Brown, A., Pardo, C. A., Zhang, Y., Becker, K. G., Wilson, B. A., de, A. L. A. P., Scharfstein, J. & Dumler, J. S., 2009.** Protease activated receptor signaling is required for African trypanosome traversal of human brain microvascular endothelial cells. *PLoS Neglected Tropical Diseases*, **3** (7): e479.

Department of Pediatrics, The Johns Hopkins University School of Medicine, Baltimore, Maryland, USA. [dgrab1@jhmi.edu].

Using human brain microvascular endothelial cells (HBMECs) as an *in vitro* model for how African trypanosomes cross the human blood-brain barrier (BBB), we recently reported

that the parasites cross the BBB by generating calcium activation signals in HBMECs through the activity of parasite cysteine proteases, particularly cathepsin L (brucipain). In the current study, we examined the possible role of a class of protease stimulated HBMEC G protein coupled receptors (GPCRs) known as protease activated receptors (PARs) that might be implicated in calcium signaling by African trypanosomes. Using RNA interference (RNAi) we found that *in vitro* PAR-2 gene (F2RL1) expression in HBMEC monolayers could be reduced by over 95 percent. We also found that the ability of *Trypanosoma brucei rhodesiense* to cross F2RL1-silenced HBMEC monolayers was reduced (39 percent-49 percent) and that HBMECs silenced for F2RL1 maintained control levels of barrier function in the presence of the parasite. Consistent with the role of PAR-2, we found that HBMEC barrier function was also maintained after blockade of Galpha(q) with *Pasteurella multocida* toxin (PMT). PAR-2 signaling has been shown in other systems to have neuroinflammatory and neuroprotective roles and our data implicate a role for proteases (i.e. brucipain) and PAR-2 in African trypanosome/HBMEC interactions. Using gene-profiling methods to interrogate candidate HBMEC pathways specifically triggered by brucipain, several pathways that potentially link some pathophysiologic processes associated with CNS HAT were identified. Together, the data support a role, in part, for GPCRs as molecular targets for parasite proteases that lead to the activation of Galpha(q)-mediated calcium signaling. The consequence of these events is predicted to be increased permeability of the BBB to parasite transmigration and the initiation of neuroinflammation, events precursory to CNS disease.

15137. **Guther, M. L., Beattie, K., Lamont, D. J., James, J., Prescott, A. R. & Ferguson, M. A., 2009.** Fate of glycosylphosphatidylinositol (GPI)-less procyclin and characterization of sialylated non-GPI-anchored surface coat molecules of procyclic-form *Trypanosoma brucei*. *Eukaryotic Cell*, **8** (9): 1407-1417.

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

A *Trypanosoma brucei* TbGPI12 null mutant that is unable to express cell surface procyclins and free glycosylphosphatidylinositols (GPI) revealed that these are not the only surface coat molecules of the procyclic life cycle stage. Here, we show that non-GPI-anchored procyclins are N-glycosylated, accumulate in the lysosome, and appear as proteolytic fragments in the medium. We also show, using lectin agglutination and galactose oxidase-NaB(3)H(4) labeling, that the cell surface of the TbGPI12 null parasites contains glycoconjugates that terminate in sialic acid linked to galactose. Following desialylation, a high-apparent-molecular-weight glycoconjugate fraction was purified by ricin affinity chromatography and gel filtration and shown to contain mannose, galactose, N-acetylglucosamine, and fucose. The latter has not been previously reported in *T. brucei* glycoproteins. A proteomic analysis of this fraction revealed a mixture of polytopic transmembrane proteins, including P-type ATPase and vacuolar proton-translocating pyrophosphatase. Immunolocalization studies showed that both could be labeled on the surfaces of wild-type and TbGPI12 null cells. Neither galactose oxidase-NaB(3)H(4) labeling of the non-GPI-anchored surface glycoconjugates nor immunogold labeling of the P-type ATPase was affected by the presence of procyclins in the wild-type cells, suggesting that the procyclins do not, by themselves, form a macromolecular barrier.

15138. **Haenni, S., Studer, E., Burkard, G. S. & Roditi, I., 2009.** Bidirectional silencing of RNA polymerase I transcription by a strand switch region in *Trypanosoma brucei*. *Nucleic Acids Research*, **37** (15): 5007-5018.

Institute of Cell Biology, University of Bern, Bern, Switzerland.

The procyclin genes in *Trypanosoma brucei* are transcribed by RNA polymerase I as part of 5-10 kb long polycistronic transcription units on chromosomes VI and X. Each procyclin locus begins with two procyclin genes followed by at least one procyclin-associated gene (PAG). In procyclic (insect midgut) form trypanosomes, PAG mRNA levels are about 100-fold lower than those of procyclins. We show that deletion of PAG1, PAG2 or PAG3 results in increased mRNA levels from downstream genes in the same transcription unit. Nascent RNA analysis revealed that most of the effects are due to increased transcription elongation in the knockouts. Furthermore, transient and stable transfections showed that sequence elements on both strands of PAG1 can inhibit Pol I transcription. Finally, by database mining we identified 30 additional PAG-related sequences that are located almost exclusively at strand switch regions and/or at sites where a change of RNA polymerase type is likely to occur.

15139. **Hanrahan, O., Webb, H., O'Byrne, R., Brabazon, E., Treumann, A., Sunter, J. D., Carrington, M. & Voorheis, H. P., 2009.** The glycosylphosphatidylinositol-PLC in *Trypanosoma brucei* forms a linear array on the exterior of the flagellar membrane before and after activation. *PLoS Pathogens*, **5** (6): e1000468.

School of Biochemistry and Immunology, Trinity College Dublin, Dublin, Ireland.

Bloodstream forms of *Trypanosoma brucei* contain a glycosylphosphatidylinositol-specific phospholipase C (GPI-PLC) that cleaves the GPI-anchor of the variable surface glycoprotein (VSG). Its location in trypanosomes has been controversial. Here, using confocal microscopy and surface labelling techniques, we show that the GPI-PLC is located exclusively in a linear array on the outside of the flagellar membrane, close to the flagellar attachment zone, but does not co-localize with the flagellar attachment zone protein, FAZ1. Consequently, the GPI-PLC and the VSG occupy the same plasma membrane leaflet, which resolves the topological problem associated with the cleavage reaction if the VSG and the GPI-PLC were on opposite sides of the membrane. The exterior location requires the enzyme to be tightly regulated to prevent VSG release under basal conditions. During stimulated VSG release in intact cells, the GPI-PLC did not change location, suggesting that the release mechanism involves lateral diffusion of the VSG in the plane of the membrane to the fixed position of the GPI-PLC.

15140. **Helm, J. R., Hertz-Fowler, C., Aslett, M., Berriman, M., Sanders, M., Quail, M. A., Soares, M. B., Bonaldo, M. F., Sakurai, T., Inoue, N. & Donelson, J. E., 2009.** Analysis of expressed sequence tags from the four main developmental stages of *Trypanosoma congolense*. *Molecular and Biochemical Parasitology*, **168** (1): 34-42.

Department of Biochemistry, Carver College of Medicine, University of Iowa,
Iowa City, IA 52242, USA.

Trypanosoma congolense is one of the most economically important pathogens of livestock in Africa. Culture-derived parasites of each of the three main insect stages of the *T. congolense* life cycle, i.e., the procyclic, epimastigote and metacyclic stages, and bloodstream stage parasites isolated from infected mice, were used to construct stage-specific cDNA libraries and expressed sequence tags (ESTs or cDNA clones) in each library were sequenced. Thirteen EST clusters encoding different variant surface glycoproteins (VSGs) were detected in the metacyclic library and 26 VSG EST clusters were found in the bloodstream library, 6 of which are shared by the metacyclic library. Rare VSG ESTs are present in the epimastigote library, and none were detected in the procyclic library. ESTs encoding enzymes that catalyze oxidative phosphorylation and amino acid metabolism are about twice as abundant in the procyclic and epimastigote stages as in the metacyclic and bloodstream stages. In contrast, ESTs encoding enzymes involved in glycolysis, the citric acid cycle and nucleotide metabolism are about the same in all four developmental stages. Cysteine proteases, kinases and phosphatases are the most abundant enzyme groups represented by the ESTs. All four libraries contain *T. congolense*-specific expressed sequences not present in the *Trypanosoma brucei* and *Trypanosoma cruzi* genomes. Normalized cDNA libraries were constructed from the metacyclic and bloodstream stages, and found to be further enriched for *T. congolense*-specific ESTs. Given that cultured *T. congolense* offer an experimental advantage over other African trypanosome species, these ESTs provide a basis for further investigation of the molecular properties of these four developmental stages, especially the epimastigote and metacyclic stages for which it is difficult to obtain large quantities of organisms. The *T. congolense* EST databases are available at: http://www.sanger.ac.uk/Projects/T_congolense/EST_index.shtml. The sequence data have been submitted to EMBL under the following accession numbers: FN263376-FN292969.

15141. **Heras, S. R., Thomas, M. C., Macias, F., Patarroyo, M. E., Alonso, C. & Lopez, M. C., 2009.** Nucleic-acid-binding properties of the C2-L1Tc nucleic acid chaperone encoded by L1Tc retrotransposon. *Biochemical Journal*, **424** (3): 479-490.

Departamento de Biología Molecular, Instituto de Parasitología y Biomedicina
Lopez Neyra, CSIC, 18001 Granada, Spain.

15142. **Hong, Y. & Kinoshita, T., 2009.** Trypanosome glycosylphosphatidylinositol biosynthesis. *Korean Journal of Parasitology*, **47** (3): 197-204.

Department of Parasitology, Kyungpook National University School of
Medicine, Daegu, Korea. [yehong@knu.ac.kr].

Trypanosoma brucei, a protozoan parasite, causes sleeping sickness in humans and Nagana disease in domestic animals in central Africa. The trypanosome surface is extensively covered by glycosylphosphatidylinositol (GPI)-anchored proteins known as variant surface glycoproteins and procyclins. GPI anchoring is suggested to be important for trypanosome survival and establishment of infection. Trypanosomes are not only pathogenically important,

but also constitute a useful model for elucidating the GPI biosynthesis pathway. This review focuses on the trypanosome GPI biosynthesis pathway. Studies on GPI that will be described indicate the potential for the design of drugs that specifically inhibit trypanosome GPI biosynthesis.

15143. **Horn, D., 2009.** Antigenic variation: extending the reach of telomeric silencing. *Current Biology*, **19** (12): R496-498.

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

Immune evasion in the parasitic African trypanosome relies upon the silencing of variant surface glycoprotein genes that are found adjacent to telomeres. Work on the RAPI telomere-binding protein now indicates that silencing spreads over a sufficient distance to repress these genes.

15144. **Ibrahim, B. S., Kanneganti, N., Rieckhof, G. E., Das, A., Laurents, D. V., Palenchar, J. B., Bellofatto, V. & Wah, D. A., 2009.** Structure of the C-terminal domain of transcription factor IIB from *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences U S A*, **106** (32): 13242-13247.

Public Health Research Institute and Department of Biochemistry and Molecular Biology, New Jersey Medical School, University of Medicine & Dentistry of New Jersey, 225 Warren Street, Newark, NJ 07103, USA.

15145. **Izquierdo, L., Schulz, B. L., Rodrigues, J. A., Guther, M. L., Procter, J. B., Barton, G. J., Aebi, M. & Ferguson, M. A., 2009.** Distinct donor and acceptor specificities of *Trypanosoma brucei* oligosaccharyltransferases. *Embo Journal*, **28** (17): 2650-2661.

Division of Biological Chemistry and Drug Discovery, The College of Life Sciences, University of Dundee, Dundee, UK.

Asparagine-linked glycosylation is catalysed by oligosaccharyltransferase (OTase). In *Trypanosoma brucei* OTase activity is catalyzed by single-subunit enzymes encoded by three paralogous genes of which TbSTT3B and TbSTT3C can complement a yeast Deltast3 mutant. The two enzymes have overlapping but distinct peptide acceptor specificities, with TbSTT3C displaying an enhanced ability to glycosylate sites flanked by acidic residues. TbSTT3A and TbSTT3B, but not TbSTT3C, are transcribed in the bloodstream and procyclic life cycle stages of *T. brucei*. Selective knockdown and analysis of parasite protein N-glycosylation showed that TbSTT3A selectively transfers biantennary Man(5)GlcNAc(2) to specific glycosylation sites whereas TbSTT3B selectively transfers triantennary Man(9)GlcNAc(2) to others. Analysis of *T. brucei* glycosylation site occupancy showed that TbSTT3A and TbSTT3B glycosylate sites in acidic to neutral and neutral to basic regions of polypeptide, respectively. This embodiment of distinct specificities in single-subunit OTases may have implications for recombinant glycoprotein engineering. TbSTT3A and TbSTT3B could be knocked down individually, but not collectively, in tissue culture. However, both

were independently essential for parasite growth in mice, suggesting that inhibiting protein N-glycosylation could have therapeutic potential against trypanosomosis.

15146. **Jensen, B. C., Sivam, D., Kifer, C. T., Myler, P. J. & Parsons, M., 2009.** Widespread variation in transcript abundance within and across developmental stages of *Trypanosoma brucei*. *BMC Genomics*, **10**: 482.

Seattle Biomedical Research Institute, 307 Westlake Ave. North, Seattle, WA 98109, USA. [bryan.jensen@sbri.org].

Trypanosoma brucei, the causative agent of African sleeping sickness, undergoes a complex developmental cycle that takes place in mammalian and insect hosts and is accompanied by changes in metabolism and cellular morphology. While differences in mRNA expression have been described for many genes, genome-wide expression analyses have been largely lacking. Trypanosomatids represent a unique case in eukaryotes in that they transcribe protein-coding genes as large polycistronic units, and rarely regulate gene expression at the level of transcription initiation. Here we present a comprehensive analysis of mRNA expression in several stages of parasite development. Utilizing microarrays that have multiple copies of multiple probes for each gene, we were able to demonstrate with a high degree of statistical confidence that approximately one-fourth of genes show differences in mRNA expression levels in the stages examined. These include complex patterns of gene expression within gene families, including the large family of variant surface glycoproteins (VSGs) and their relatives, where we have identified a number of constitutively expressed family members. Furthermore, we were able to assess the relative abundance of all transcripts in each stage, identifying the genes that are either weakly or highly expressed. Very few genes show no evidence of expression. Despite the lack of gene regulation at the level of transcription initiation, our results reveal extensive regulation of mRNA abundance associated with different life cycle and growth stages. In addition, analysis of variant surface glycoprotein gene expression reveals a more complex picture than previously thought. These data provide a valuable resource to the community of researchers studying this lethal agent.

15147. **Kabani, S., Fenn, K., Ross, A., Ivens, A., Smith, T. K., Ghazal, P. & Matthews, K., 2009.** Genome-wide expression profiling of *in vivo*-derived bloodstream parasite stages and dynamic analysis of mRNA alterations during synchronous differentiation in *Trypanosoma brucei*. *BMC Genomics*, **10**: 427.

Centre for Immunity, Infection and Evolution, Institute of Immunology and Infection Research, School of Biological Sciences, King's Buildings, University of Edinburgh, Edinburgh, UK. [S.Kabani@sms.ed.ac.uk].

Trypanosomes undergo extensive developmental changes during their complex life cycle. Crucial among these is the transition between slender and stumpy bloodstream forms and, thereafter, the differentiation from stumpy to tsetse-midgut procyclic forms. These developmental events are highly regulated, temporally reproducible and accompanied by expression changes mediated almost exclusively at the post-transcriptional level. In this study we have examined, by whole-genome microarray analysis, the mRNA abundance of genes in slender and stumpy forms of *T. brucei* AnTat1.1 cells, and also during their synchronous differentiation to procyclic forms. In total, five biological replicates representing the

differentiation of matched parasite populations derived from five individual mouse infections were assayed, with RNAs being derived at key biological time points during the time course of their synchronous differentiation to procyclic forms. Importantly, the biological context of these mRNA profiles was established by assaying the coincident cellular events in each population (surface antigen exchange, morphological restructuring, cell cycle re-entry), thereby linking the observed gene expression changes to the well-established framework of trypanosome differentiation. Using stringent statistical analysis and validation of the derived profiles against experimentally-predicted gene expression and phenotypic changes, we have established the profile of regulated gene expression during these important life-cycle transitions. The highly synchronous nature of differentiation between stumpy and procyclic forms also means that these studies of mRNA profiles are directly relevant to the changes in mRNA abundance within individual cells during this well-characterized developmental transition.

15148. **Koopmann, R., Muhammad, K., Perbandt, M., Betzel, C. & Duszenko, M., 2009.** *Trypanosoma brucei* ATG8: structural insights into autophagic-like mechanisms in protozoa. *Autophagy*, **5** (8): 1085-1091.

Interfaculty Institute for Biochemistry, University of Tübingen, Tübingen, Germany.

15149. **Koslowsky, D. J., 2009.** Complex interactions in the regulation of trypanosome mitochondrial gene expression. *Trends in Parasitology*, **25** (6): 252-255.

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI 48824, USA.

Trypanosomes undergo extreme physiological changes to adapt to different environments as they cycle between hosts. Adaptation to the different environments has evolved an energy metabolism involving a mitochondrion with an unusual genome. Recently, Aphasizhev and colleagues have identified two new protein complexes, a mitochondrial polyadenylation complex and a guide RNA stabilization complex, that provide novel insights into the coordinated expression of the mitochondrial genome.

15150. **Kuettel, S., Mosimann, M., Maser, P., Kaiser, M., Brun, R., Scapozza, L. & Perozzo, R., 2009.** Adenosine kinase of *T. b. rhodesiense* identified as the putative target of 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]morpholine using chemical proteomics. *PLoS Neglected Tropical Diseases*, **3** (8): e506.

Pharmaceutical Biochemistry Group, School of Pharmaceutical Sciences, University of Lausanne, Geneva, Switzerland.

Human African trypanosomosis (HAT), a major parasitic disease spread in Africa, urgently needs novel targets and new efficacious chemotherapeutic agents. Recently, we discovered that 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]morpholine (compound 1) exhibits specific antitrypanosomal activity with an IC₅₀ of 1.0 μM on *Trypanosoma brucei rhodesiense* (*T. b. rhodesiense*), the causative agent of the acute form of HAT. In this work we show adenosine kinase of *T. b. rhodesiense* (TbrAK), a key enzyme of the parasite purine

salvage pathway which is vital for parasite survival, to be the putative intracellular target of compound 1 using a chemical proteomics approach. This finding was confirmed by RNA interference experiments showing that down-regulation of adenosine kinase counteracts compound 1 activity. Further chemical validation demonstrated that compound 1 interacts specifically and tightly with TbrAK with nanomolar affinity, and *in vitro* activity measurements showed that compound 1 is an enhancer of TbrAK activity. The subsequent kinetic analysis provided strong evidence that the observed hyperactivation of TbrAK is due to the abolishment of the intrinsic substrate-inhibition. The results suggest that TbrAK is the putative target of this compound, and that hyperactivation of TbrAK may represent a novel therapeutic strategy for the development of trypanocides.

15151. **Kunz, S., Luginbuehl, E. & Seebeck, T., 2009.** Gene conversion transfers the GAF-A domain of phosphodiesterase TbrPDEB1 to one allele of TbrPDEB2 of *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases*, **3** (6): e455.

Institute of Cell Biology, University of Bern, Bern, Switzerland.

15152. **Lacomble, S., Portman, N. & Gull, K., 2009.** A protein-protein interaction map of the *Trypanosoma brucei* paraflagellar rod. *PLoS One*, **4** (11): e7685.

Sir William Dunn School of Pathology and Oxford Centre for Integrative Systems Biology, University of Oxford, Oxford, UK.

We have conducted a protein interaction study of components within a specific sub-compartment of a eukaryotic flagellum. The trypanosome flagellum contains a paracrystalline extra-axonemal structure termed the paraflagellar rod (PFR) with around forty identified components. We have used a Gateway cloning approach coupled with yeast two-hybrid, RNAi and 2D DiGE to define a protein-protein interaction network taking place in this structure. We define two clusters of interactions; the first being characterised by two proteins with a shared domain which is not sufficient for maintaining the interaction. The other cohort is populated by eight proteins, a number of which possess a PFR domain and sub-populations of this network exhibit dependency relationships. Finally, we provide clues as to the structural organization of the PFR at the molecular level. This multi-strand approach shows that protein interactome data can be generated for insoluble protein complexes.

15153. **Li, Z., Umeyama, T. & Wang, C. C., 2009.** The aurora kinase in *Trypanosoma brucei* plays distinctive roles in metaphase-anaphase transition and cytokinetic initiation. *PLoS Pathogens*, **5** (9): e1000575.

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

Aurora B kinase is an essential regulator of chromosome segregation with the action well characterized in eukaryotes. It is also implicated in cytokinesis, but the detailed mechanism remains less clear, partly due to the difficulty in separating the latter from the former function in a growing cell. A chemical genetic approach with an inhibitor of the enzyme added to a synchronized cell population at different stages of the cell cycle would probably solve this problem. In the deeply branched parasitic protozoan *Trypanosoma brucei*,

an aurora B homologue, TbAUK1, was found to control both chromosome segregation and cytokinetic initiation by evidence from RNAi and dominant negative mutation. To clearly separate these two functions, VX-680, an inhibitor of TbAUK1, was added to a synchronized *T. brucei* procyclic cell population at different cell cycle stages. The unique trans-localization pattern of the chromosomal passenger complex (CPC), consisting of TbAUK1 and two novel proteins TbCPC1 and TbCPC2, was monitored during mitosis and cytokinesis by following the migration of the proteins tagged with enhanced yellow fluorescence protein in live cells with time-lapse video microscopy. Inhibition of TbAUK1 function in S-phase, prophase or metaphase invariably arrests the cells in the metaphase, suggesting an action of TbAUK1 in promoting metaphase-anaphase transition. TbAUK1 inhibition in anaphase does not affect mitotic exit, but prevents trans-localization of the CPC from the spindle midzone to the anterior tip of the new flagellum attachment zone for cytokinetic initiation. The CPC in the midzone is dispersed back to the two segregated nuclei, while cytokinesis is inhibited. In and beyond telophase, TbAUK1 inhibition has no effect on the progression of cytokinesis or the subsequent G1, S and G2 phases until a new metaphase is attained. There are thus two clearly distinct points of TbAUK1 action in *T. brucei*: the metaphase-anaphase transition and cytokinetic initiation. This is the first time to our knowledge that the dual functions of an Aurora B homologue are dissected and separated into two clearly distinct time frames in a cell cycle.

15154. **Liu, B., Wang, J., Yildirim, G. & Englund, P. T., 2009.** TbPIF5 is a *Trypanosoma brucei* mitochondrial DNA helicase involved in processing of minicircle Okazaki fragments. *PLoS Pathogens*, **5** (9): e1000589.

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

15155. **Luz Ambrosio, D., Lee, J. H., Panigrahi, A. K., Nguyen, T. N., Cicarelli, R. M. & Gunzl, A., 2009.** Spliceosomal proteomics in *Trypanosoma brucei* reveal new RNA splicing factors. *Eukaryotic Cell*, **8** (7): 990-1000.

Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, Connecticut 06030-3301, USA.

15156. **Manful, T., Cristodero, M. & Clayton, C., 2009.** DRBD1 is the *Trypanosoma brucei* homologue of the spliceosome-associated protein 49. *Molecular and Biochemical Parasitology*, **166** (2): 186-189.

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), ZMBH-DKFZ Alliance, Im Neuenheimer Feld 282, D69120 Heidelberg, Germany.

15157. **Mao, Y., Najafabadi, H. S. & Salavati, R., 2009.** Genome-wide computational identification of functional RNA elements in *Trypanosoma brucei*. *BMC Genomics*, **10**: 355.

Institute of Parasitology, McGill University, Montreal, Quebec H9X3V9, Canada. [yuan.mao@mail.mcgill.ca].

Post-transcriptional regulation of gene expression is the dominant regulatory mechanism in trypanosomatids as their mRNAs are transcribed from polycistronic units. A few cis-acting RNA elements in 3'-untranslated regions of mRNAs have been identified in trypanosomatids, which affect the mRNA stability or translation rate in different life stages of these parasites. Other functional RNAs (fRNAs) also play essential roles in these organisms. However, there has been no genome-wide analysis for identification of fRNAs in trypanosomatids. Functional RNAs, including non-coding RNAs (ncRNAs) and cis-acting RNA elements involved in post-transcriptional gene regulation, were predicted based on two independent computational analyses of the genome of *Trypanosoma brucei*. In the first analysis, the predicted candidate ncRNAs were identified based on conservation with the related trypanosomatid *Leishmania braziliensis*. This prediction had a substantially low estimated false discovery rate, and a considerable number of the predicted ncRNAs represented novel classes with unknown functions. In the second analysis, we identified a number of function-specific regulatory motifs, based on which we devised a classifier that can be used for homology-independent function prediction in *T. brucei*. This first genome-wide analysis of fRNAs in trypanosomatids restricts the search space of experimental approaches and, thus, can significantly expedite the process of characterization of these elements. Our classifier for function prediction based on cis-acting regulatory elements can also, in combination with other methods, provide the means for homology-independent annotation of trypanosomatid genomes.

15158. **Marciano, D., Maugeri, D. A., Cazzulo, J. J. & Nowicki, C., 2009.** Functional characterization of stage-specific aminotransferases from trypanosomatids. *Molecular and Biochemical Parasitology*, **166** (2): 172-182.

Instituto de Química y Físicoquímica Biológica IQUIFIB-CONICET, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junin 956, C1113AAD Buenos Aires, Argentina.

15159. **Marechal, A., Kido, Y., Kita, K., Moore, A. L. & Rich, P. R., 2009.** Three redox states of *Trypanosoma brucei* alternative oxidase identified by infrared spectroscopy and electrochemistry. *Journal of Biological Chemistry*, **284** (46): 31827-31833.

Glynn Laboratory of Bioenergetics, Institute of Structural and Molecular Biology, University College London, Gower Street, London WC1E 6BT, UK.

15160. **Mattiacio, J. L. & Read, L. K., 2009.** Evidence for a degradosome-like complex in the mitochondria of *Trypanosoma brucei*. *FEBS Letters*, **583** (14): 2333-2338.

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

15161. **McCulloch, R. & Horn, D., 2009.** What has DNA sequencing revealed about the VSG expression sites of African trypanosomes? *Trends in Parasitology*, **25** (8): 359-363.

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

Antigenic variation is crucial for the survival of African trypanosomes in mammals and involves switches in expression of variant surface glycoprotein genes, which are co-transcribed with a number of expression-site-associated genes (ESAGs) from loci termed “bloodstream expression sites” (BESs). Trypanosomes possess multiple BESs, although the reason for this (and why ESAGs are resident in these loci) has remained a subject of debate. The genome sequence of *Trypanosoma brucei*, released in 2005, did not include the BESs because of their telomeric disposition. This gap in our knowledge has now been bridged by two new studies, which we discuss here, asking what has been revealed about the biological significance of BES multiplicity and ESAG function and evolution.

15162. **Monnerat, S., Clucas, C., Brown, E., Mottram, J. C. & Hammarton, T. C., 2009.** Searching for novel cell cycle regulators in *Trypanosoma brucei* with an RNA interference screen. *BMC Research Notes*, **2**: 46.

Division of Infection & Immunity, Faculty of Biomedical and Life Sciences and Wellcome Centre for Molecular Parasitology, University of Glasgow, Glasgow, UK. [severine.monnerat@sbri.org].

The protozoan parasite, *Trypanosoma brucei*, is spread by the tsetse fly and causes Human African Trypanosomosis. Its cell cycle is complex and not fully understood at the molecular level. The *T. brucei* genome contains over 6 000 protein coding genes with >50 percent having no predicted function. A small scale RNA interference (RNAi) screen was carried out in *Trypanosoma brucei* to evaluate the prospects for identifying novel cycle regulators. Procytic form *T. brucei* was transfected with a genomic RNAi library and 204 clones isolated. However, only 76 RNAi clones were found to target a protein coding gene of potential interest. These clones were screened for defects in proliferation and cell cycle progression following RNAi induction. Sixteen clones exhibited proliferation defects upon RNAi induction, with eight clones displaying potential cell cycle defects. To confirm the phenotypes, new RNAi cell lines were generated and characterised for five genes targeted in these clones. While we confirmed that the targeted genes are essential for proliferation, we were unable to unambiguously classify them as cell cycle regulators. Our study identified genes essential for proliferation, but did not, as hoped, identify novel cell cycle regulators. Screening of the RNAi library for essential genes was extremely labour-intensive, which was compounded by the suboptimal quality of the library. For such a screening method to be viable for a large scale or genome wide screen, a new, significantly improved RNAi library will be required, and automated phenotyping approaches will need to be incorporated.

15163. **Morrison, L. J., Tait, A., McLellan, S., Sweeney, L., Turner, C. M. & MacLeod, A., 2009.** A major genetic locus in *Trypanosoma brucei* is a determinant of host pathology. *PLoS Neglected Tropical Diseases*, **3** (12): e557.

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

The progression and variation of pathology during infections can be due to components from both host or pathogen, and/or the interaction between them. The influence of host genetic variation on disease pathology during infections with trypanosomes has been well studied in recent years, but the role of parasite genetic variation has not been extensively studied. We have shown that there is parasite strain-specific variation in the level of splenomegaly and hepatomegaly in infected mice and used a forward genetic approach to identify the parasite loci that determine this variation. This approach allowed us to dissect and identify the parasite loci that determine the complex phenotypes induced by infection. Using the available trypanosome genetic map, a major quantitative trait locus (QTL) was identified on *T. brucei* chromosome 3 (LOD = 7.2) that accounted for approximately two thirds of the variance observed in each of two correlated phenotypes, splenomegaly and hepatomegaly, in the infected mice (named TbOrg1). In addition, a second locus was identified that contributed to splenomegaly, hepatomegaly and reticulocytosis (TbOrg2). This is the first use of quantitative trait locus mapping in a diploid protozoan and shows that there are trypanosome genes that directly contribute to the progression of pathology during infections and, therefore, that parasite genetic variation can be a critical factor in disease outcome. The identification of parasite loci is a first step towards identifying the genes that are responsible for these important traits and shows the power of genetic analysis as a tool for dissecting complex quantitative phenotypic traits.

15164. **Morriswood, B., He, C. Y., Sealey-Cardona, M., Yelinek, J., Pypaert, M. & Warren, G., 2009.** The bilobe structure of *Trypanosoma brucei* contains a MORN-repeat protein. *Molecular and Biochemical Parasitology*, **167** (2): 95-103.

Max F. Perutz Laboratories, University of Vienna, Medical University of Vienna, Dr. Bohr-Gasse 9, A-1030 Vienna, Austria.

The Golgi of the kinetoplastid parasite *Trypanosoma brucei* is closely apposed to a bilobe structure containing TbCentrin2 and TbCentrin4 in procyclic cells. However, both are additionally localized to the basal bodies. Here we report the characterization of a membrane occupation and recognition nexus (MORN)-repeat protein, TbMORN1, present at the bilobe but not at the basal body. The anterior part of the TbMORN1 structure partially overlapped with the flagellar attachment zone while the posterior part overlapped with the flagellar pocket. Depletion studies using RNAi showed that there was a modest growth inhibition in procyclic cells but lethality in bloodstream cells, showing that it is an essential protein in the bloodstream form of the organism. TbMORN1 appears to be a useful marker for the bilobe in *T. brucei*.

15165. **Natesan, S. K., Peacock, L., Leung, K. F., Matthews, K. R., Gibson, W. & Field, M. C., 2009.** The trypanosome Rab-related proteins RabX1 and RabX2 play no role in intracellular trafficking but may be involved in fly infectivity. *PLoS One*, **4** (9): e7217.

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

Rab GTPases constitute the largest subgroup of the Ras superfamily and are primarily involved in vesicle targeting. The full extent of Rab family function is unexplored. Several divergent Rab-like proteins are known but few have been characterized. In *Trypanosoma*

brucei there are sixteen Rab genes, but RabX1, RabX2 and RabX3 are divergent within canonical sequence regions. Where known, trypanosome Rab functions are broadly conserved when orthologous relationships may be robustly established, but specific functions for RabX1, X2 and X3 have yet to be determined. RabX1 and RabX2 originated via tandem duplication and subcellular localization places RabX1 at the endoplasmic reticulum, while RabX2 is at the Golgi complex, suggesting distinct functions. We wished to determine whether RabX1 and RabX2 are involved in vesicle transport or other cellular processes. Using comparative genomics we find that RabX1 and RabX2 are restricted to trypanosomatids. Gene knockout indicates that RabX1 and RabX2 are non-essential. Simultaneous RNAi knockdown of both RabX1 and RabX2, while partial, was also non-lethal and may suggest non-redundant function, consistent with the distinct locations of the proteins. Analysis of the knockout cell lines unexpectedly failed to uncover a defect in exocytosis, endocytosis or in the morphology or location of multiple markers for the endomembrane system, suggesting that neither RabX1 nor RabX2 has a major role in intracellular transport. However, it was apparent that RabX1 and RabX2 knockout cells displayed somewhat enhanced survival within flies. RabX1 and RabX2, two members of the trypanosome Rab subfamily, were shown to have no major detectable role in intracellular transport, despite the localization of each gene product to highly specific endomembrane compartments. These data extend the functional scope of Rab proteins in trypanosomes to include non-canonical roles in differentiation-associated processes in protozoa.

15166. **Olego-Fernandez, S., Vaughan, S., Shaw, M. K., Gull, K. & Ginger, M. L., 2009.** Cell morphogenesis of *Trypanosoma brucei* requires the paralogous, differentially expressed calpain-related proteins CAP5.5 and CAP5.5V. *Protist*, **160** (4): 576-590.

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

15167. **Osato, D., Rogers, K., Guo, Q., Li, F., Richmond, G., Klug, F. & Simpson, L., 2009.** Uridine insertion/deletion RNA editing in trypanosomatid mitochondria: In search of the editosome. *Rna*, **15** (7): 1338-1344.

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

15168. **Ouellette, M. & Papadopoulou, B., 2009.** Coordinated gene expression by post-transcriptional regulons in African trypanosomes. *Journal of Biology*, **8** (11): 100.

Centre de Recherche en Infectiologie et Département de Microbiologie-Infectiologie et Immunologie, Université Laval, Quebec, G1V 4G2, Canada. [Marc.Ouellette@crchul.ulaval.ca.].

15169. **Padilla-Mejia, N. E., Florencio-Martinez, L. E., Figueroa-Angulo, E. E., Manning-Cela, R. G., Hernandez-Rivas, R., Myler, P. J. & Martinez-Calvillo, S., 2009.** Gene organization and sequence analyses of transfer RNA genes in Trypanosomatid parasites. *BMC Genomics*, **10**: 232.

Unidad de Biomedicina, Facultad de Estudios Superiores Iztacala, Universidad Nacional Autonoma de Mexico, Av de los Barrios 1, Col Los Reyes Iztacala, Tlalnepanla, Edo de Mexico, Mexico. [qfedime@yahoo.com.mx].

15170. **Palfi, Z., Jae, N., Preusser, C., Kaminska, K. H., Bujnicki, J. M., Lee, J. H., Gunzl, A., Kambach, C., Urlaub, H. & Bindereif, A., 2009.** SMN-assisted assembly of snRNP-specific Sm cores in trypanosomes. *Genes and Development*, **23** (14): 1650-1664.

Institute of Biochemistry, Justus Liebig University of Giessen, Giessen, Germany.

15171. **Patrick, K. L., Shi, H., Kolev, N. G., Ersfeld, K., Tschudi, C. & Ullu, E., 2009.** Distinct and overlapping roles for two Dicer-like proteins in the RNA interference pathways of the ancient eukaryote *Trypanosoma brucei*. *Proceedings of the National Academy of Sciences U S A*, **106** (42): 17933-17938.

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

Trypanosoma brucei is one of the most ancient eukaryotes where RNA interference (RNAi) is operational and is the only single-cell pathogen where RNAi has been extensively studied and used as a tool for functional analyses. Here, we report that the *T. brucei* RNAi pathway, although relying on a single Argonaute protein (AGO1), is initiated by the activities of two distinct Dicer-like enzymes. Both TbDCL1, a mostly cytoplasmic protein, and the previously undescribed nuclear enzyme TbDCL2 contribute to the biogenesis of siRNAs from retroposons. However, TbDCL2 has a predominant role in generating siRNAs from chromosomal internal repeat transcripts that accumulate at the nucleolus in RNAi-deficient cells and in initiating the endogenous RNAi response against retroposons and repeats alike. Moreover, siRNAs generated by both TbDCL1 and TbDCL2 carry a 5'-monophosphate and a blocked 3' terminus, suggesting that 3' end modification is an ancient trait of siRNAs. We thus propose a model whereby TbDCL2 fuels the *T. brucei* nuclear RNAi pathway and TbDCL1 patrols the cytoplasm, posttranscriptionally silencing potentially harmful nucleic acid parasites that may access the cytoplasm. Nevertheless, we also provide evidence for cross-talk between the two Dicer-like enzymes, because TbDCL2 is implicated in the generation of 35- to 65-nucleotide intermediate transcripts that appear to be substrates for TbDCL1. Our finding that dcl2KO cells are more sensitive to RNAi triggers than wild-type cells has significant implications for reverse genetic analyses in this important human pathogen.

15172. **Poliak, P., Van Hoewyk, D., Obornik, M., Zikova, A., Stuart, K. D., Tachezy, J., Pilon, M. & Lukes, J.,** Functions and cellular localization of cysteine desulfurase and selenocysteine lyase in *Trypanosoma brucei*. *Febs Journal*, **277** (2): 383-393.

Biology Centre, Institute of Parasitology and Faculty of Science, University of South Bohemia, Ceske Budejovice (Budweis), Czech Republic.

Nfs-like proteins have cysteine desulfurase (CysD) activity, which removes sulphur (S) from cysteine, and provides S for iron-sulphur cluster assembly and the thiolation of tRNAs. These proteins also have selenocysteine lyase activity *in vitro*, and cleave selenocysteine into alanine and elemental selenium (Se). It was shown previously that the Nfs-like protein called Nfs from the parasitic protist *Trypanosoma brucei* is a genuine CysD. A second Nfs-like protein is encoded in the nuclear genome of *T. brucei*. We called this protein selenocysteine lyase (SCL) because phylogenetic analysis reveals that it is monophyletic with known eukaryotic selenocysteine lyases. The Nfs protein is located in the mitochondrion, whereas the SCL protein seems to be present in the nucleus and cytoplasm. Unexpectedly, downregulation of either Nfs or SCL protein leads to a dramatic decrease in both CysD and selenocysteine lyase activities concurrently in the mitochondrion and the cytosolic fractions. Because loss of Nfs causes a growth phenotype but loss of SCL does not, we propose that Nfs can fully complement SCL, whereas SCL can only partially replace Nfs under our growth conditions.

15173. **Preusser, C., Palfi, Z. & Bindereif, A., 2009.** Special Sm core complex functions in assembly of the U2 small nuclear ribonucleoprotein of *Trypanosoma brucei*. *Eukaryotic Cell*, **8** (8): 1228-1234.

Institute of Biochemistry, Justus Liebig University of Giessen, Germany.

15174. **Queiroz, R., Benz, C., Fellenberg, K., Hoheisel, J. D. & Clayton, C., 2009.** Transcriptome analysis of differentiating trypanosomes reveals the existence of multiple post-transcriptional regulons. *BMC Genomics*, **10**: 495.

Zentrum für Molekulare Biologie der Universität Heidelberg, ZMBH-DKFZ Alliance, Im Neuenheimer Feld 282, 69120 Heidelberg, Germany. [r.queiroz@dkfz-heidelberg.de].

Trypanosome gene expression is regulated almost exclusively at the post-transcriptional level, with mRNA degradation playing a decisive role. When trypanosomes are transferred from the blood of a mammal to the midgut of a tsetse fly, they transform to procyclic forms: gene expression is reprogrammed, changing the cell surface and switching the mode of energy metabolism. Within the blood, trypanosomes can pre-adapt for tsetse transmission, becoming growth-arrested stumpy forms. We describe here the transitions in gene expression that occur during differentiation of *in-vitro* cultured bloodstream forms to procyclic forms. Some mRNAs showed changes within 30 min of cis-aconitate addition, whereas others responded 12-24 h later. For the first 12 h after addition of cis-aconitate, cells accumulated at the G1 phase of the cell cycle, and showed decreases in mRNAs required for proliferation, mimicking the changes seen in stumpy forms: many mRNAs needed for ribosomal and flagellar biogenesis showed striking co-regulation. Other mRNAs encoding components of signal transduction pathways and potential regulators were specifically induced only during differentiation. Messenger RNAs encoding proteins required for individual metabolic pathways were often co-regulated. It is concluded that trypanosome genes form post-transcriptional regulons in which mRNAs with functions in particular pathways, or encoding components of protein complexes, show almost identical patterns of regulation.

15175. **Ralston, K. S., Kabututu, Z. P., Melehani, J. H., Oberholzer, M. & Hill, K. L., 2009.** The *Trypanosoma brucei* flagellum: moving parasites in new directions. *Annual Review of Microbiology*, **63**: 335-362.

Department of Microbiology, Immunology, and Molecular Genetics, University of California, Los Angeles, California 90095, USA.

African trypanosomes are devastating human and animal pathogens. *Trypanosoma brucei rhodesiense* and *T. b. gambiense* subspecies cause the fatal human disease known as African sleeping sickness. It is estimated that several hundred thousand new infections occur annually and the disease is fatal if untreated. *T. brucei* is transmitted by the tsetse fly and alternates between bloodstream-form and insect-form life cycle stages that are adapted to survive in the mammalian host and the insect vector, respectively. The importance of the flagellum for parasite motility and attachment to the tsetse fly salivary gland epithelium has been appreciated for many years. Recent studies have revealed both conserved and novel features of *T. brucei* flagellum structure and composition, as well as surprising new functions that are outlined here. These discoveries are important from the standpoint of understanding trypanosome biology and identifying novel drug targets, as well as for advancing our understanding of fundamental aspects of eukaryotic flagellum structure and function.

15176. **Ramey, K., Eko, F. O., Thompson, W. E., Armah, H., Igietseme, J. U. & Stiles, J. K., 2009.** Immunolocalization and challenge studies using a recombinant *Vibrio cholerae* ghost expressing *Trypanosoma brucei* Ca²⁺ ATPase (TBCA2) antigen. *American Journal of Tropical Medicine and Hygiene*, **81** (3): 407-415.

Department of Microbiology, Biochemistry and Immunology, Morehouse School of Medicine, Atlanta, Georgia 30310, USA. [kramey@msm.edu].

Human African trypanosomosis is a neglected disease caused by *Trypanosoma brucei* spp. A parasite cation pump (Ca²⁺ ATPase; TBCA2) essential for survival and cation homeostasis was identified and characterized. It was hypothesized that targeting this pump using a *Vibrio cholerae* ghost (VCG)-based vaccine could protect against murine *T. brucei* infection. mRNA and protein expression of TBCA2 was differentially expressed in blood and insect stages of parasites and immunolocalized in the pericellular membrane and the flagellar pocket of bloodstream forms. Antigen-specific antibodies and Th1 cytokines, interleukin-2, interferon-gamma, and tumor necrosis factor-alpha were induced in rVCG-TBCA2-immunized mice and *in vitro* on antigen stimulation of splenic immune T cells, but the corresponding Th2-type response was unremarkable. Despite an increased median survival of six days in vaccinated mice, the mice were not protected against infection. Thus, immunization of mice produced robust parasite-specific antibodies but failed to protect mice against parasite challenge.

15177. **Reynolds, T. B., 2009.** Strategies for acquiring the phospholipid metabolite inositol in pathogenic bacteria, fungi and protozoa: making it and taking it. *Microbiology*, **155** (5): 1386-1396.

Department of Microbiology, University of Tennessee, Knoxville, TN 37996, USA. [treynol6@utk.edu].

Myo-inositol (inositol) is an essential nutrient that is used for building phosphatidylinositol and its derivatives in eukaryotes and even in some eubacteria such as the mycobacteria. As a consequence, fungal, protozoan and mycobacterial pathogens must be able to acquire inositol in order to proliferate and cause infection in their hosts. There are two primary mechanisms for acquiring inositol. One is to synthesize inositol from glucose 6-phosphate using two sequentially acting enzymes: inositol-3-phosphate synthase (Ino1p) converts glucose 6-phosphate to inositol 3-phosphate, and then inositol monophosphatase (IMPase) dephosphorylates inositol 3-phosphate to generate inositol. The other mechanism is to import inositol from the environment via inositol transporters. Inositol is readily abundant in the bloodstream of mammalian hosts, providing a source from which many pathogens could potentially import inositol. However, despite this abundance of inositol in the host, some pathogens such as the bacterium *Mycobacterium tuberculosis* and the protist parasite *Trypanosoma brucei* must be able to make inositol *de novo* in order to cause disease (*M. tuberculosis*) or even grow (*T. brucei*). Other pathogens such as the fungus *Candida albicans* are equally adept at causing disease by importing inositol or by making it *de novo*. The role of inositol acquisition in the biology and pathogenesis of the parasite *Leishmania* and the fungus *Cryptococcus* are being explored as well. The specific strategies used by these pathogens to acquire inositol while in the host are discussed in relation to each pathogen's unique metabolic requirements.

15178. Riviere, L., Moreau, P., Allmann, S., Hahn, M., Biran, M., Plazolles, N., Franconi, J. M., Boshart, M. & Bringaud, F., 2009. Acetate produced in the mitochondrion is the essential precursor for lipid biosynthesis in procyclic trypanosomes. *Proceedings of the National Academy of Sciences U S A*, **106** (31): 12694-12699.

Laboratoire de Microbiologie Cellulaire et Moléculaire et Pathogénicité,
Unité Mixte de Recherche 5234 Centre National de la Recherche Scientifique,
Université Victor Segalen Bordeaux 2, 146 rue Leo Saignat, 33076 Bordeaux
cedex, France.

Acetyl-CoA produced in mitochondria from carbohydrate or amino acid catabolism needs to reach the cytosol to initiate *de novo* synthesis of fatty acids. All eukaryotes analyzed so far use a citrate/malate shuttle to transfer acetyl group equivalents from the mitochondrial matrix to the cytosol. Here we investigate how this acetyl group transfer occurs in the procyclic life cycle stage of *Trypanosoma brucei*, a protozoan parasite responsible of human sleeping sickness and economically important livestock diseases. Deletion of the potential citrate lyase gene, a critical cytosolic enzyme of the citrate/malate shuttle, has no effect on *de novo* biosynthesis of fatty acids from ¹⁴C-labeled glucose, indicating that another route is used for acetyl group transfer. Because acetate is produced from acetyl-CoA in the mitochondrion of this parasite, we considered genes encoding cytosolic enzymes producing acetyl-CoA from acetate. We identified an acetyl-CoA synthetase gene encoding a cytosolic enzyme (AceCS), which is essential for cell viability. Repression of AceCS by inducible RNAi results in a 20-fold reduction of ¹⁴C-incorporation from radiolabeled glucose or acetate into *de novo* synthesized fatty acids. Thus, we demonstrate that the essential cytosolic enzyme AceCS of *T. brucei* is responsible for activation of acetate into acetyl-CoA to feed *de novo* biosynthesis of lipids. To date, *Trypanosoma* is the only known eukaryotic organism

that uses acetate instead of citrate to transfer acetyl groups over the mitochondrial membrane for cytosolic lipid synthesis.

15179. **Rodriguez, J. A., Lopez, M. A., Thayer, M. C., Zhao, Y., Oberholzer, M., Chang, D. D., Kisalu, N. K., Penichet, M. L., Helguera, G., Bruinsma, R., Hill, K. L. & Miao, J., 2009.** Propulsion of African trypanosomes is driven by bihelical waves with alternating chirality separated by kinks. *Proceedings of the National Academy of Sciences U S A*, 106 (46): 19322-19327.

Department of Surgery, Division of Surgical Oncology, Molecular Biology Institute, The David Geffen School of Medicine, University of California, Los Angeles, CA 90095, USA.

Trypanosoma brucei, a parasitic protist with a single flagellum, is the causative agent of African sleeping sickness. Propulsion of *T. brucei* was long believed to be by a drill-like, helical motion. Using millisecond differential interference-contrast microscopy and analyzing image sequences of cultured procyclic-form and bloodstream-form parasites, as well as bloodstream-form cells in infected mouse blood, we find that, instead, motility of *T. brucei* is by the propagation of kinks, separating left-handed and right-handed helical waves. Kink-driven motility, previously encountered in prokaryotes, permits *T. brucei* a helical propagation mechanism while avoiding the large viscous drag associated with a net rotation of the broad end of its tapering body. Our study demonstrates that millisecond differential interference-contrast microscopy can be a useful tool for uncovering important short-time features of microorganism locomotion.

15180. **Sauvage, V., Aubert, D., Escotte-Binet, S. & Villena, I., 2009.** The role of ATP-binding cassette (ABC) proteins in protozoan parasites. *Molecular and Biochemical Parasitology*, 167 (2): 81-94.

Laboratoire de Parasitologie-Mycologie, EA 3800, IFR 53, UFR Médecine, Université de Reims Champagne-Ardenne, 51 rue Cognacq-Jay, 51095 Reims Cedex, France.

The ATP-binding cassette (ABC) superfamily is one of the largest protein families with representatives in all kingdoms of life. Members of this superfamily are involved in a wide variety of transport processes with substrates ranging from small ions to relatively large polypeptides and polysaccharides, but also in cellular processes such as DNA repair, translation or regulation of gene expression. For many years, the role of ABC proteins was mainly investigated for their implication in drug resistance. However, recent studies focused rather on their physiological functions for the parasite. In this review, we present an overview of ABC proteins in major protozoan parasites including *Leishmania*, *Trypanosoma*, *Plasmodium*, *Toxoplasma*, *Cryptosporidium* and *Entamoeba* species. We will also discuss the role of characterized ABC transporters in the biology of the parasite and in drug resistance.

15181. **Schwede, A., Manful, T., Jha, B. A., Helbig, C., Bercovich, N., Stewart, M. & Clayton, C., 2009.** The role of deadenylation in the degradation of unstable mRNAs in trypanosomes. *Nucleic Acids Research*, 37 (16): 5511-5528.

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

15182. **Sevova, E. S. & Bangs, J. D., 2009.** Streamlined architecture and glycosylphosphatidylinositol-dependent trafficking in the early secretory pathway of African trypanosomes. *Molecular Biology of the Cell*, **20** (22): 4739-4750.

Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53706, USA.

The variant surface glycoprotein (VSG) of bloodstream form *Trypanosoma brucei* is a critical virulence factor. The VSG glycosylphosphatidylinositol (GPI)-anchor strongly influences passage through the early secretory pathway. Using a dominant-negative mutation of TbSar1, we show that endoplasmic reticulum (ER) exit of secretory cargo in trypanosomes is dependent on the coat protein complex II (COPII) machinery. Trypanosomes have two orthologues each of the Sec23 and Sec24 COPII subunits, which form specific heterodimeric pairs: TbSec23.1/TbSec24.2 and TbSec23.2/TbSec24.1. RNA interference silencing of each subunit is lethal but has minimal effects on trafficking of soluble and transmembrane proteins. However, silencing of the TbSec23.2/TbSec24.1 pair selectively impairs ER exit of GPI-anchored cargo. All four subunits colocalize to one or two ER exit sites (ERES), in close alignment with the postnuclear flagellar adherence zone (FAZ), and closely juxtaposed to corresponding Golgi clusters. These ERES are nucleated on the FAZ-associated ER. The Golgi matrix protein Tb Golgi reassembly stacking protein defines a region between the ERES and Golgi, suggesting a possible structural role in the ERES:Golgi junction. Our results confirm a selective mechanism for GPI-anchored cargo loading into COPII vesicles and a remarkable degree of streamlining in the early secretory pathway. This unusual architecture probably maximizes efficiency of VSG transport and fidelity in organellar segregation during cytokinesis.

15183. **Shi, H., Chamond, N., Djikeng, A., Tschudi, C. & Ullu, E., 2009.** RNA interference in *Trypanosoma brucei*: role of the n-terminal RGG domain and the polyribosome association of argonaute. *Journal of Biological Chemistry*, **284** (52): 36511-36520.

Department of Internal Medicine, Yale University Medical School, New Haven, Connecticut 06536-8012, USA.

15184. **Singha, U. K., Sharma, S. & Chaudhuri, M., 2009.** Downregulation of mitochondrial porin inhibits cell growth and alters respiratory phenotype in *Trypanosoma brucei*. *Eukaryotic Cell*, **8** (9): 1418-1428.

Department of Microbial Pathogenesis and Immune Response, Meharry Medical College, Nashville, TN 37208, USA.

Porin is the most abundant outer membrane (OM) protein of mitochondria. It forms the aqueous channel on the mitochondrial OM and mediates major metabolite flux between mitochondria and cytosol. Mitochondrial porin in *Trypanosoma brucei*, a unicellular parasitic protozoan and the causative agent of African trypanosomosis, possesses a beta-barrel

structure similar to the bacterial OM porin OmpA. *T. brucei* porin (TbPorin) is present as a monomer as well as an oligomer on the mitochondrial OM, and its expression is developmentally regulated. In spite of its distinct structure, the TbPorin function is similar to those of other eukaryotic porins. TbPorin RNA interference (RNAi) reduced cell growth in both procyclic and bloodstream forms. The depletion of TbPorin decreased ATP production by inhibiting metabolite flux through the OM. Additionally, the level of trypanosome alternative oxidase (TAO) decreased, whereas the levels of cytochrome-dependent respiratory complexes III and IV increased in TbPorin-depleted mitochondria. Furthermore, the depletion of TbPorin reduced cellular respiration via TAO, which is not coupled with oxidative phosphorylation, but increased the capacity for cyanide-sensitive respiration. Together, these data reveal that TbPorin knockdown reduced the mitochondrial ATP level, which in turn increased the capacity of the cytochrome-dependent respiratory pathway (CP), in an attempt to compensate for the mitochondrial energy crisis. However, a simultaneous decrease in the substrate-level phosphorylation due to TbPorin RNAi caused growth inhibition in the procyclic form. We also found that the expressions of TAO and CP proteins are coordinately regulated in *T. brucei* according to mitochondrial energy demand.

15185. **Spitznagel, D., Ebikeme, C., Biran, M., Nic a' Bhaird, N., Bringaud, F., Henehan, G. T. & Nolan, D. P., 2009.** Alanine aminotransferase of *Trypanosoma brucei*-a key role in proline metabolism in procyclic life forms. *Febs Journal*, **276** (23): 7187-7199.

School of Biochemistry and Immunology, Trinity College Dublin, Ireland.

African trypanosomes possess high levels of alanine aminotransferase (EC 2.6.1.2), although the function of their activity remains enigmatic, especially in slender bloodstream forms where the metabolism of ketoacids does not occur. Therefore, the gene for alanine aminotransferase enzyme in *Trypanosoma brucei* (TbAAT) was characterized and its function assessed using a combination of RNA interference and gene knockout approaches. Surprisingly, as much as 95 percent or more of the activity appears to be unnecessary for growth of either bloodstream or procyclic forms respiring on glucose. A combination of RNA interference and NMR spectroscopy revealed an important role for the activity in procyclic forms respiring on proline. Under these conditions, the major end product of proline metabolism is alanine, and a reduction in TbAAT activity led to a proportionate decrease in the amount of alanine excreted along with an increase in the doubling time of the cells. These results provide evidence of a role for alanine aminotransferase in the metabolism of proline in African trypanosomes by linking glutamate produced by the initial oxidative steps of the pathway with pyruvate produced by the final oxidative step of the pathway. This step appears to be essential when proline is the primary carbon source, which is likely to be the physiological situation in the tsetse fly vector.

15186. **Tazeh, N. N., Silverman, J. S., Schwartz, K. J., Sevova, E. S., Sutterwala, S. S. & Bangs, J. D., 2009.** Role of AP-1 in developmentally regulated lysosomal trafficking in *Trypanosoma brucei*. *Eukaryotic Cell*, **8** (9): 1352-1361.

Department of Medical Microbiology and Immunology, University of Wisconsin School of Medicine and Public Health, Madison, WI 53706, USA.

African trypanosomes are the causative agents of human trypanosomosis (sleeping sickness). The pathogenic stage of the parasite has unique adaptations to life in the bloodstream of the mammalian host, including upregulation of endocytic and lysosomal activities. We investigated stage-specific requirements for cytoplasmic adaptor/clathrin machinery in post-Golgi apparatus biosynthetic sorting to the lysosome using RNA interference silencing of the Tbm1 subunit of adaptor complex 1 (AP-1), in conjunction with immunolocalization, kinetic analyses of reporter transport, and quantitative endocytosis assays. Tbm1 silencing was lethal in both stages, indicating a critical function(s) for the AP-1 machinery. Transport of soluble and membrane-bound secretory cargoes was Tbm1 independent in both stages. In procyclic parasites, trafficking of the lysosomal membrane protein, p67, was disrupted, leading to cell surface mislocalization. The lysosomal protease trypanopain was also secreted, suggesting a transmembrane-sorting receptor for this soluble hydrolase. In bloodstream trypanosomes, both p67 and trypanopain trafficking were unaffected by Tbm1 silencing, suggesting that AP-1 is not necessary for biosynthetic lysosomal trafficking. Endocytosis in bloodstream cells was also unaffected, indicating that AP-1 does not function at the flagellar pocket. These results indicate that post-Golgi apparatus sorting to the lysosome is critically dependent on the AP-1/clathrin machinery in procyclic trypanosomes but that this machinery is not necessary in bloodstream parasites. We propose a simple model for stage-specific default secretory trafficking in trypanosomes that is consistent with the behaviour of other soluble and glycosylphosphatidylinositol-anchored cargos and which is influenced by upregulation of endocytosis in bloodstream parasites as an adaptation to life in the mammalian bloodstream.

15187. **Thomson, R., Molina-Portela, P., Mott, H., Carrington, M. & Raper, J., 2009.** Hydrodynamic gene delivery of baboon trypanosome lytic factor eliminates both animal and human-infective African trypanosomes. *Proceedings of the National Academy of Sciences U S A*, **106** (46): 19509-19514.

Department of Medical Parasitology, New York University Langone School of Medicine, New York, NY 10010, USA.

Several species of African trypanosomes cause fatal disease in livestock, but most cannot infect humans due to innate trypanosome lytic factors (TLFs). Human TLFs are pore-forming high-density lipoprotein (HDL) particles that contain apolipoprotein L-I (apoL-I) the trypanolytic component, and haptoglobin-related protein (Hpr), which binds free haemoglobin (Hb) in blood and facilitates the uptake of TLF via a trypanosome haptoglobin-haemoglobin receptor. The human-infective *Trypanosoma brucei rhodesiense* escapes lysis by TLF by expression of serum resistance-associated (SRA) protein, which binds and neutralizes apoL-I. Unlike humans, baboons are not susceptible to infection by *T. b. rhodesiense* due to previously unidentified serum factors. Here, we show that baboons have a TLF complex that contains orthologues of Hpr and apoL-I and that full-length baboon apoL-I confers trypanolytic activity to mice and when expressed together with baboon Hpr and human apoA-I, provides protection against both animal infective and the human-infective *T. brucei rhodesiense* *in vivo*. We further define two critical lysines near the C terminus of baboon apoL-I that are necessary and sufficient to prevent binding to SRA and thereby confer resistance to human-infective trypanosomes. These findings form the basis for the creation of TLF transgenic livestock that would be resistant to animal and human-infective

trypanosomes, which would result in the reduction of disease and the zoonotic transmission of human infective trypanosomes.

15188. **Tielens, A. G. & van Hellemond, J. J., 2009.** Surprising variety in energy metabolism within Trypanosomatidae. *Trends in Parasitology*, **25** (10): 482-490.

Department of Medical Microbiology and Infectious Diseases, Erasmus MC, University Medical Centre Rotterdam, 'S Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. [a.tielens@erasmusmc.nl].

The metabolism of Trypanosomatidae differs significantly between distinct species and can even be completely different between various life-cycle stages of the same species. It has been proposed that differences in energy metabolism are related to differences in nutrient supply in the environments of the various trypanosomatids. However, the literature shows that availability of substrates does not dictate the type of energy metabolism of trypanosomatids, as *Trypanosoma theileri*, *Trypanosoma lewisi* and African trypanosomes all live in the bloodstream of their mammalian host, but have surprisingly large differences in metabolism. Furthermore, in trypanosomatids no obvious relationship exists between energy metabolism and phylogeny or mode of transmission. We provide an overview of the metabolic capacities in the energy metabolism of distinct trypanosomatids, and suggest that these can be divided into four different metabolic categories of increasing complexity.

15189. **Urbaniak, M. D., 2009.** Casein kinase 1 isoform 2 is essential for bloodstream form *Trypanosoma brucei*. *Molecular and Biochemical Parasitology*, **166** (2): 183-185.

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

Induction of RNA interference targeted against casein kinase 1 isoform 2 (TbCK1.2, Tb927.5.800) in bloodstream form *Trypanosoma brucei* *in vitro* results in rapid cessation of growth, gross morphological changes, multinucleation and ultimately cell death. A null mutant of the highly homologous casein kinase 1 isoform 1 (Tb927.5.790) in bloodstream form *T. brucei* displays no growth or morphological phenotype *in vitro*. A truncated form of TbCK1.2 expressed in *Escherichia coli* as a GST fusion produces catalytically active recombinant protein, facilitating screening for small molecule inhibitors. These data show that TbCK1.2 is an attractive target for anti-trypanosomal drug discovery.

15190. **van Grinsven, K. W., Van Den Abbeele, J., Van den Bossche, P., van Hellemond, J. J. & Tielens, A. G., 2009.** Adaptations in the glucose metabolism of procyclic *Trypanosoma brucei* isolates from tsetse flies and during differentiation of bloodstream forms. *Eukaryotic Cell*, **8** (8): 1307-1311.

Department of Biochemistry and Cell Biology, Utrecht University, The Netherlands.

Procyclic forms of *Trypanosoma brucei* isolated from the midguts of infected tsetse flies, or freshly transformed from a strain that is close to field isolates, do not use a complete

Krebs cycle. Furthermore, short stumpy bloodstream forms produce acetate and are apparently metabolically preadapted to adequate functioning in the tsetse fly.

15191. **Verstrepen, K. J. & Fink, G. R., 2009.** Genetic and epigenetic mechanisms underlying cell-surface variability in protozoa and fungi. *Annual Review of Genetics*, **43**: 1-24.

Laboratory for Systems Biology, VIB, B-3001 Leuven, Belgium.
[kevin.verstrepen@biw.vib-kuleuven.be].

Eukaryotic microorganisms have evolved ingenious mechanisms to generate variability at their cell surface, permitting differential adherence, rapid adaptation to changing environments, and evasion of immune surveillance. Fungi such as *Saccharomyces cerevisiae* and the pathogen *Candida albicans* carry a family of mucin and adhesin genes that allow adhesion to various surfaces and tissues. *Trypanosoma cruzi*, *T. brucei*, and *Plasmodium falciparum* likewise contain large arsenals of different cell surface adhesion genes. In both yeasts and protozoa, silencing and differential expression of the gene family results in surface variability. Here, we discuss unexpected similarities in the structure and genomic location of the cell surface genes, the role of repeated DNA sequences, and the genetic and epigenetic mechanisms—all of which contribute to the remarkable cell surface variability in these highly divergent microbes.

15192. **Willert, E. K. & Phillips, M. A., 2009.** Cross-species activation of trypanosome S-adenosylmethionine decarboxylase by the regulatory subunit prozyme. *Molecular and Biochemical Parasitology*, **168** (1): 1-6.

Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, 6001 Forest Park Road, Dallas, TX 75390-9041, USA.

15193. **Wyllie, S., Oza, S. L., Patterson, S., Spinks, D., Thompson, S. & Fairlamb, A. H., 2009.** Dissecting the essentiality of the bifunctional trypanothione synthetase-amidase in *Trypanosoma brucei* using chemical and genetic methods. *Molecular Microbiology*, **74** (3): 529-540.

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

The bifunctional trypanothione synthetase-amidase (TRY5) comprises two structurally distinct catalytic domains for synthesis and hydrolysis of trypanothione (N(1),N(8)-bis(glutathionyl)spermidine). This unique dithiol plays a pivotal role in thiol-redox homeostasis and in defence against chemical and oxidative stress in trypanosomatids. A tetracycline-dependent conditional double knockout of TRY5 (cDKO) was generated in bloodstream *Trypanosoma brucei*. Culture of cDKO parasites without tetracycline induction resulted in loss of trypanothione and accumulation of glutathione, followed by growth inhibition and cell lysis after six days. In the absence of inducer, cDKO cells were unable to infect mice, confirming that this enzyme is essential for virulence *in vivo* as well as *in vitro*. To establish whether both enzymatic functions were essential, an amidase-dead mutant

cDKO line was generated. In the presence of inducer, this line showed decreased growth *in vitro* and decreased virulence *in vivo*, indicating that the amidase function is not absolutely required for viability. The druggability of TRYS was assessed using a potent small molecule inhibitor developed in our laboratory. Growth inhibition correlated in rank order cDKO, single KO, wild-type and overexpressing lines and produced the predicted biochemical phenotype. The synthetase function of TRYS is thus unequivocally validated as a drug target by both chemical and genetic methods.

15194. **Zamudio, J. R., Mittra, B., Campbell, D. A. & Sturm, N. R., 2009.** Hypermethylated cap 4 maximizes *Trypanosoma brucei* translation. *Molecular Microbiology*, **72** (5): 1100-1110.

Department of Microbiology, Immunology and Molecular Genetics, David Geffen School of Medicine, University of California at Los Angeles, Los Angeles, CA 90095-1489, USA.

Through trans-splicing of a 39-nt spliced leader (SL) onto each protein-coding transcript, mature kinetoplastid mRNA acquire a hypermethylated 5'-cap structure, but its function has been unclear. Gene deletions for three *Trypanosoma brucei* cap 2'-O-ribose methyltransferases, TbMTr1, TbMTr2 and TbMTr3, reveal distinct roles for four 2'-O-methylated nucleotides. Elimination of individual gene pairs yields viable cells; however, attempts at double knock-outs resulted in the generation of a TbMTr2-/-/TbMTr3-/- cell line only. Absence of both kinetoplastid-specific enzymes in TbMTr2-/-/TbMTr3-/- lines yielded substrate SL RNA and mRNA with cap 1. TbMTr1-/- translation is comparable with wildtype, while cap 3 and cap 4 loss reduced translation rates, exacerbated by the additional loss of cap 2. TbMTr1-/- and TbMTr2-/-/TbMTr3-/- lines grow to lower densities under normal culture conditions relative to wildtype cells, with growth rate differences apparent under low serum conditions. Cell viability may not tolerate delays at both the nucleolar Sm-independent and nucleoplasmic Sm-dependent stages of SL RNA maturation combined with reduced rates of translation. A minimal level of mRNA cap ribose methylation is essential for trypanosome viability, providing the first functional role for the cap 4.

15195. **Zikova, A., Schnauffer, A., Dalley, R. A., Panigrahi, A. K. & Stuart, K. D., 2009.** The F(0)F(1)-ATP synthase complex contains novel subunits and is essential for procyclic *Trypanosoma brucei*. *PLoS Pathogens*, **5** (5): e1000436.

Seattle Biomedical Research Institute, Seattle, WA, USA.

The mitochondrial F(0)F(1) ATP synthase is an essential multi-subunit protein complex in the vast majority of eukaryotes but little is known about its composition and role in *Trypanosoma brucei*, an early diverged eukaryotic pathogen. We purified the F(0)F(1) ATP synthase by a combination of affinity purification, immunoprecipitation and blue-native gel electrophoresis and characterized its composition and function. We identified 22 proteins of which five are related to F(1) subunits, three to F(0) subunits, and 14 which have no obvious homology to proteins outside the kinetoplastids. RNAi silencing of expression of the F(1) alpha subunit or either of the two novel proteins showed that they are each essential for the viability of procyclic (insect stage) cells and are important for the structural integrity of the F(0)F(1)-ATP synthase complex. We also observed a dramatic decrease in ATP production

by oxidative phosphorylation after silencing expression of each of these proteins while substrate phosphorylation was not severely affected. Our procyclic *T. brucei* cells were sensitive to the ATP synthase inhibitor oligomycin even in the presence of glucose contrary to earlier reports. Hence, the two novel proteins appear essential for the structural organization of the functional complex and regulation of mitochondrial energy generation in these organisms is more complicated than previously thought.

ISBN 978-92-5-206641-5 ISSN 1812-2450



9 789252 066415

11736F/1/08.10