year **2012**
volume **35**
part **1**

PAAT

Programme Against African Trypanosomosis

ISSN 1812-2442

TSETSE AND TRYPANOSOMOSIS INFORMATION

DFID
Department for International Development

CIRAD

year **2012**

volume **35**

part **1**

PAAT

Programme
Against
African
Trypanosomosis

TSETSE AND TRYPANOSOMOSIS INFORMATION

Numbers 16037-16293

FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS
Rome, 2012

ISBN 978-92-5-107434-3

TSETSE AND TRYPANOSOMOSIS INFORMATION

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

The half-yearly periodical is prepared for publication, in both English and French editions, by the Food and Agriculture Organization of the United Nations. Each annual volume consists of two parts and an index. Subscription is free for all recipients engaged in trypanosomosis research and control, and requests for enrolment may be sent to: Ms Maria Grazia Solari, AGAH, FAO, Viale delle Terme di Caracalla, 00100 Rome, Italy (fax +39 06 5705 5749; e-mail MariaGrazia.Solari@fao.org).

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

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

Distribution dates and copy deadlines

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

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

ABBREVIATIONS USED IN TTI

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

Organizations

ANDE	Agence Nationale de Développement de l'Elevage
AU	African Union
AU/STRC	African Union/Scientific, Technical and Research Commission
BICOT	Biological Control of Tsetse by the Sterile Insect Technique
CEBV	Communauté Economique du Bétail et de la Viande
CEMV	Centre Universitaire de Formation en Entomologie Médicale et Vétérinaire
CGIAR	Consultative Group on International Agricultural Research
CIRAD	Centre de Coopération Internationale en Recherche Agronomique pour le Développement
CIRAD-EMVT	Département d'Elevage et de Médecine Vétérinaire des Pays Tropicaux du CIRAD
CIRDES	Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide
CNERV	Centre National d'Elevage et de Recherches Vétérinaires
CNRS	Centre National de Recherche Scientifique
CREAT	Centre de Recherche et d'Elevage, Avétonou, Togo
CRSSA	Centre de Recherches du Service de Santé des Armées Emile Pardé
CTVM	Centre for Tropical Veterinary Medicine
DFID	Department for International Development (UK)
DNDi	Drugs for Neglected Diseases Initiative
DSE	German Foundation for International Development
EC/EU	European Community/European Union
EDF	European Development Fund
FAO	Food and Agriculture Organization of the United Nations
FITCA	Farming in Tsetse Control Areas of Eastern Africa

GTZ	Deutsche Gesellschaft für Technische Zusammenarbeit
IAEA	International Atomic Energy Agency
IBAR	Interafrican Bureau for Animal Resources
ICIPE	International Centre of Insect Physiology and Ecology
ICPTV	Integrated Control of Pathogenic Trypanosomes and their Vectors
IFAD	International Fund for Agricultural Development
ILRI	International Livestock Research Institute
INRA	Institut National de Recherche Agronomique
IPR	Institut Pierre Richet
IRD	Institut de Recherche et de Développement (formerly ORSTOM)
ISCTRC	International Scientific Council for Trypanosomiasis Research and Control
ISRA	Institut Sénégalais de Recherches Agricoles
ITC	International Trypanotolerance Centre
KARI	Kenya Agricultural Research Institute
KETRI	Kenya Trypanosomiasis Research Institute
LCV	Laboratoire Central Vétérinaire
LNERV	Laboratoire National de l'Elevage et de Recherches Vétérinaires
LSHTM	London School of Hygiene and Tropical Medicine
MRC	Medical Research Council
MRU	Mano River Union
NITR	Nigerian Institute for Trypanosomiasis Research
NRI	Natural Resources Institute
OCCGE Grande	Organisation de Coopération et de Coordination pour la Lutte contre les Endémies
OCEAC	Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale
OGAPROV	Office Gabonais pour l'Amélioration de la Production de la Viande
OIE	Office International des Epizooties
OMVG	Organisation pour la Mise en Valeur du Fleuve Gambie
PAAT	Programme against African Trypanosomosis
PATTEC	Pan-African Tsetse and Trypanosomiasis Eradication Campaign
PRCT	Projet de Recherches Cliniques sur la Trypanosomiase
RDI	Rural Development International
RUCA	Rijksuniversitair Centrum Antwerpen
SADC	Southern African Development Community
SIDA	Swedish International Development Authority
SODEPRA	Société pour le Développement des Productions Animales
TDR	UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases
TDRC	Tropical Diseases Research Centre
TPRI	Tropical Pesticides Research Institute
TTRI	Tsetse and Trypanosomiasis Research Institute
UNDP	United Nations Development Programme
USAID	United States Agency for International Development
USDA	United States Department of Agriculture
UTRO	Uganda Trypanosomiasis Research Organisation
WHO	World Health Organization

CONTENTS

SECTION A – NEWS

MEMORANDUM OF UNDERSTANDING BETWEEN THE AFRICAN UNION COMMISSION AND THE FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

The following is the text of the above, signed on behalf of FAO by Ms. Maria H. Semedo, Assistant Director General and Regional Representative for Africa, and by H.E. Mrs Rhoda P. Tumusiime, Commissioner, Rural Economy and Agriculture, on behalf of the Commission of the African Union.

PREAMBLE

Considering that the African Heads of State and Government Summit held in Lomé, Togo in July 2000, adopted Decision AHG/ Dec. 56 (XXXVI) urging Member States to collectively embark on a Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC); and charged the Commission of the African Union (hereinafter referred to as AUC), of initiating and coordinating activities aimed at the eradication of trypanosomosis through mobilizing and organizing phased intervention action in the affected countries;

Whereas the Food and Agriculture Organization of the United Nations (hereinafter referred to as FAO), carries out activities on animal health and production in developing countries, and is one of the partner institutions of the Programme Against African Trypanosomosis (PAAT) established by FAO Conference Resolution 5/97 of 17 November 1997;

Bearing in mind that FAO and other United Nations (UN) system agencies, which over the years have been involved in efforts to mobilize and coordinate activities aimed at increasing the control of animal African trypanosomosis based on the principle of integrated pest management (IPM) and within the context of sustainable agriculture and rural development (SARD). In their commitment to implement the objectives of the PATTEC Initiative, several countries have initiated activities on the ground or developed plans aimed at integrated tsetse and trypanosomosis control;

Whereas within the context of their respective mandates, roles and common objectives, and based on their shared interest to work more concertedly through consolidating, rationalizing and harmonizing their contribution in support of the efforts in affected Member States in their bid to reduce and ultimately eliminate the burden of tsetse-transmitted trypanosomosis to SARD;

The Commission and FAO (hereinafter jointly referred to as "the Parties" and in the singular as "a Party"), who are mandated by their respective Member States to lead, mobilize, organize and coordinate efforts in the initiation, promotion, support and implementation of activities aimed at fighting African trypanosomosis, have agreed to strengthen their collaboration, enhance their respective roles and strengthen their collective action through formalizing their cooperation.

Therefore the Parties have agreed to enter into the following Memorandum of Understanding (hereinafter referred to as the "MOU"):

Article 1

Objectives

1. The overall objective of this Memorandum of Understanding is to formalize the collaboration between the Commission and FAO in the identification of the modalities for improving cooperation and coordination of activities in respect to matters of common interest in the Parties' efforts to address the tsetse and trypanosomosis problem.

2. The Parties agree that they will act in close cooperation and consult each other in regard to matters of common interest, aim at international harmonization and synergized contributions by relevant partners whenever this may be appropriate in light of their respective mandates.

Article 2

Institutional arrangements

1. The Parties shall establish full transparency and communication channels to facilitate and enhance cooperation between the Commission and FAO while avoiding overlap or duplication in exercising their respective mandates.

2. The Parties shall appoint a Focal Point responsible for the coordination of activities as laid out in Articles 3 and 4 below of the present Memorandum of Understanding.

Article 3

Areas of cooperation

1. The Commission and FAO shall cooperate at international, regional and national levels and shall, within their respective mandates, explore possibilities for effective joint action in conformity with their respective rules, regulations, procedures and administrative practices in the following areas:

(a) Preparation and presentation of documents, reports and proposals, as appropriate;
(b) Preparation of plans and project proposals aimed at tsetse and trypanosomosis intervention;
(c) Involvement in activities prepared or being undertaken by the other Party, such as training courses, workshops, planning, monitoring and evaluation of projects;
(d) Contact and communication with tsetse and trypanosomosis affected countries in sub-Saharan Africa and third parties on issues related to tsetse and trypanosomosis intervention;
(e) Dissemination of information about collaborative activities, goals and objectives;
(f) FAO shall provide technical support to the Commission in the planning, execution, monitoring and evaluation of PATTEC projects;
(g) The Parties shall engage in regular consultations and shall actively participate in bilateral and other meetings including those of the Programme Against African Trypanosomosis (PAAT) and events related to the cooperation under this Memorandum of Understanding, subject to the respective Party's rules and practices with regard to meetings and events.

Article 4

Specific areas of cooperation

1. Each particular cooperation activity shall be agreed to by the Parties on a case-by-case basis. Specific agreements shall be concluded between the Parties whereby roles and obligations of the Parties for each particular cooperation activity shall be reflected.
2. Each Party shall implement its activities under its sole control and shall be responsible for the implementation of its own activities.
3. The Parties, subject to their respective mandates, financial regulations and rules, policies and procedures, agree to cooperate in specific areas including the following:

(a) Assistance in training and capacity development activity;
(b) Applied research, methods development and validation to address technical gaps and bottlenecks and to improve the efficiency and cost-effectiveness of operational field projects and interventions, particularly, but not exclusively, in subjects related to general animal health, parasitology, Geographic Information System applications for risk assessment, disease treatments, quality control of interventions, sustainable agriculture and rural development, land use and socio-economy;
(c) Mutual participation in relevant policy coordination, planning, research and other meetings, workshops and events;
(d) Mutual continuous exchanging of data and information subject to their confidentiality obligations;
(e) Assistance in the development of national and regional legislation and regulatory measures;
(f) Sharing of reports and publications of mutual interest;
(g) Mutually support of each other's programmes at resource mobilization events.

Article 5

Review of the cooperation

The Parties shall meet once a year at a mutually agreed date and location to discuss their collaboration under this Memorandum of Understanding.

Article 6

Financial arrangements

Nothing in this Memorandum of Understanding shall give rise to legal or financial obligations upon either Party. Where measures taken to implement this Memorandum of Understanding may give rise to any financial or legal obligations, the Parties shall conclude a separate agreement, subject to the AUC's and the FAO Financial Rules and Regulations, prior to such measures being undertaken.

Article 7

Personnel

Any personnel employed by the Parties shall remain subject to the rules and regulations of their respective institutions in all matters of employment, medical and life insurance and employee rights and benefits.

Article 8

Dissemination of information

The Commission and FAO shall support the widest possible dissemination of unclassified information provided or exchanged under this Memorandum of Understanding, subject to the need to protect proprietary information. The Commission and FAO shall ensure the confidentiality of information classified by the other party as restricted or confidential.

Article 9

Privileges and immunities

Nothing in this Memorandum of Understanding or in any document or arrangement relating thereto, shall be construed as constituting a waiver of privileges or immunities of the Parties, nor as conferring any privileges or immunities of one Party to the other Party or to its personnel.

Article 10

Intellectual property

Intellectual property rights, in particular copyright, of material such as information, software and designs, made available by the Commission and FAO to be used to carry out the activities under this Memorandum of Understanding shall remain with the originating Party.

Copyright of the information, as well as rights to any other intellectual property, developed jointly by the Commission and FAO shall be jointly vested in the Parties.

Article 11

Use of name, emblem or official seal

1. FAO shall not use the name, emblem or official seal of the Commission and PATTEC for any purpose other than expressly authorized in writing by the Commission.

2. The Commission shall not use the name, emblem or official seal of FAO and PAAT for any purpose other than expressly authorized in writing by FAO.

Article 12

Dispute Settlement

Any dispute between the Parties arising out of or relating to the interpretation or implementation of the present Memorandum of Understanding shall be finally amicably settled through negotiations or by such means, as the Parties may mutually agree on.

Article 13

Amendment

The provisions of the present Memorandum of Understanding may be modified by written agreement between the Parties. Any such modification shall enter into force thirty (30) days from the date of such written agreement, or where such agreement is made by exchange of letters, from the date of the later letter.

Article 14

Termination

This Memorandum of Understanding may be terminated by either Party upon three (3)-month written notice given to the other Party. In that event, the Parties shall agree on measures required for the orderly conclusion of ongoing activities. In the absence of written notice by one Party to the other of non-renewal, the present Memorandum of Understanding shall be automatically renewable for further three (3)-year periods.

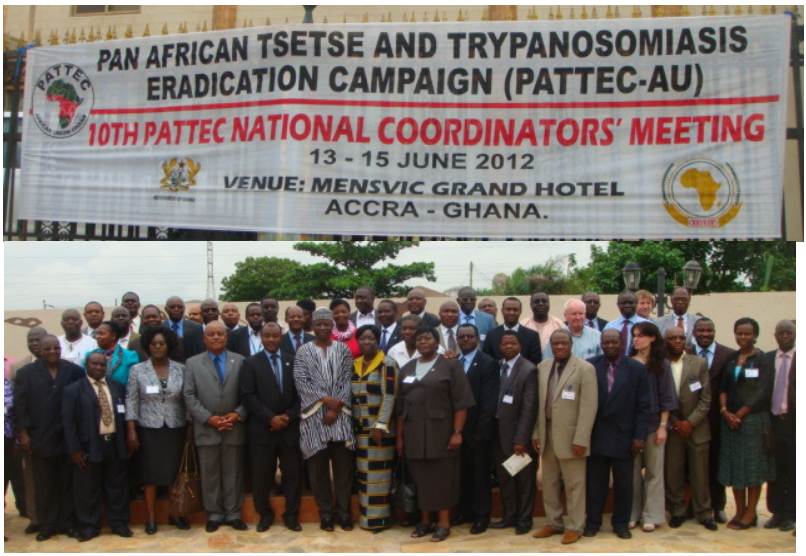
Article 15

Entry into force

This Memorandum of Understanding shall enter into force upon signature by the Parties. Where signature takes place on two different dates, the present Memorandum of Understanding shall enter into force from the date of the second signature.

IN WITNESS WHEREOF, the duly authorized representatives of the African Union Commission and the Food and Agriculture Organization of the United Nations, have hereby signed the present Memorandum of Understanding in two (2) original copies in the English language.

10TH PATTEC NATIONAL COORDINATORS' MEETING, ACCRA, GHANA, 13-15 JUNE 2012



Group photograph of the participants to the meeting

The10th PATTEC National Coordinators' meeting opened on 13 June 2012 at the Mensvic Grand Hotel in Accra, Republic of Ghana. The meeting brought together about 90 national PATTEC Coordinators and focal points from 26 African countries, representatives of international organizations, institutions of higher learning, private and public partners including, the Office International des Epizooties (OIE), International Atomic Energy Agency (IAEA), Food and Agriculture Organization of the United Nations (FAO), AU-Inter African Bureau for Animal Resources (AU-IBAR), Global Alliance for Livestock Veterinary Medicines (GALVmed), National Resources Institute – UK (NRI), Institut de Recherche pour le Développement (IRD), Organisation de Coordination pour la lutte contre les Grandes Endémies en Afrique Centrale (OCEAC), Centre International de Recherche-Développement Sur l'Elevage en Zone Subhumide (CIRDES), Swiss Tropical Public Health Institute (STHI), University of Neuchatel, Leverhume Trust Tsetse Research Network (LTTRN), Tanzania Trypanosomiasis Research Institute (TTRI), Tanzania National Park (TANAPA), Tanzania Wildlife Research Institute (TAWIRI), Kenya Agricultural Research Institute-Trypanosomiasis Research Center (KARI-TRC) among others.

Speaking at the opening ceremony; Dr Daniel Bourzat of the OIE representing the Director General reported on the relation of the OIE and the AU. He reiterated the support of the OIE to the implementation of the PATTEC initiative.

IAEA, represented by Dr Udo Feldmann reported that the IAEA General Conference passed a new resolution to support PATTEC. He further informed the participants of the support provided to PATTEC and T&T affected countries.

Dr Oumar Diall on behalf of FAO reported on the support of FAO to tsetse and trypanosomosis countries and the PATTEC Coordination Unit. He reiterated the commitments of the FAO to work closely with PATTEC. On the other hand, Dr Merixtell Donadue of GALVmed presented her organization and the trypanosomiasis project, which is funded by DFID to deal with animal trypanosomiasis. She reiterated GALVmed support to the implementation of PATTEC.

Mr Kwesi Quartey, Ambassador of the Republic of Ghana to Ethiopia and Permanent representative to the African Union and the Economic Commission for Africa said that as a member of the Permanent Representative Committee attending a PATTEC' meeting for the first time, he was positively impressed by the presence of so many PATTEC national coordinators, focal points, development partners etc. who showed commitment to work together to eliminate the scourge of tsetse and trypanosomiasis from Africa. Dr Abebe Haile Gabriel, Director of DREA in his opening statement made it clear to the participants that PATTEC should be part of the greater picture, which is the Comprehensive Agricultural Development Programme (CAADP). He requested representatives of affected countries present at the meeting to include PATTEC activities in their CAADP programmes.

H.E. Dr Alfred Tia Sugri, Deputy Minister for Food and Agriculture in charge of Livestock informed the participants in his opening statement about the gains made by his country's project after the first phase of the Multinational Regional Project for the creation of tsetse and trypanosomiasis free areas in East and West Africa. He said Ghana will continue to advocate for greater collaboration and the sharing of ideas and experiences in science and technology for the betterment of its people.

According to the representative of the African Development Bank, Mr Karikari, the meeting was an opportunity to review the progress of implementation of the on-going projects, identify key challenges, share lessons learned and agree on ways forward and action plans. He further assured participants that the recommendations of this meeting will receive all the requisite attention from the World Bank.

Dr Hassane H. Mahamat, AU-PATTEC Coordinator in his presentation of the PATTEC report, concurred with the Director of the Department of Rural Economy and Agriculture that once the PATTEC Initiative is well implemented, it will play an important role in all four CAADP Pillars. The PATTEC Coordinator also discussed lessons learned, challenges and the way forward for the implementation of PATTEC.

The bureau of the meeting was elected after the opening ceremony as follows: Dr Charles Mahama, National PATTEC Ghana was elected Chairman, assisted by Dr Hassane H. Mahamat, AU-PATTEC Coordinator. Dr Yahaya Adam from Ghana, Seth Onyango from Kenya, Mr. Christian Hazoume and Girma Urgeacha from the AU-PATTEC Coordination Office were elected as rapporteurs.

Representatives of five out of the six countries namely; Ghana, Kenya, Uganda, Burkina Faso and Mali - all countries funded by the African Development Bank within the framework of the multinational project for the creation of tsetse and trypanosomiasis free areas in East and West Africa gave brief overviews of the status of the implementation of their PATTEC projects. Ghana and Kenya, presented their project completion reports and the ways forward. Burkina Faso and Mali, informed the participants that their respective projects had been extended until the end of 2013. The representative from Uganda presented the country's future plans on T&T.

The meeting continued the following day with presentations from the following partners/stakeholders: OIE, IBAR/ISCTRC, CIRDES, the Vector Group represented by NRI and IRD, OCEAC, University of Neuchatel, TRC, TTRI, etc. who described their respective

organizations, their mandates and visions as well as their activities. The OIE representative explained to the participants how a disease such as trypanosomiasis can be listed on the OIE list.

The situation report of the following countries with regard to ongoing tsetse and trypanosomiasis project activities, plans and programmes as well as their efforts to mobilize resources were presented. These included: Angola, Botswana, Cameroon, Chad, Congo, DR Congo, Equatorial Guinea, Gabon, Guinea-Conakry, Malawi, Niger, Nigeria, Senegal, Sierra Leone, South Sudan, Sudan, Tanzania, Togo, Zambia, and Zimbabwe.

During the working session with industry partners on their support to the implementation of the PATTEC Initiative, Vestergaard-Frandsen and Orsmonds made presentations about their respective companies. Vestergaard-Frandsen presented the Zerofly® net and the benefits of its use. Orsmonds aviation presented how the process of preparing a plane for SAT operations is being done and the difficulties associated with this.

The meeting proceeded on the third day (15 June 2012) with the presentations of regional projects in the Central Africa region (Chad, CAR, Cameroon and Nigeria); South-Eastern Africa region (Zimbabwe, Zambia, Malawi and Mozambique) and West Africa region (Nigeria, Niger, Burkina Faso, Togo, and Benin). During the session, the PATTEC Coordination Office presented a template for a questionnaire on M&E and GIS data management.

Dr Assefa Mebrate presented the PATTEC Strategic Plan for the Period 2012 – 2017 while Dr Francis Oloo presented guidelines for tsetse and trypanosomosis control. Dr Fabrizio Tediosi and Peter Steinman of the Swiss Tropical and Health Institute presented the project "Eradication Investment Cases for Onchocerciasis, Lymphatic Filariasis and Human African Trypanosomiasis".

Recommendations

1. The Meeting noted the implementation of the PATTEC Initiative by affected countries; the Meeting noted that the benefits of successful implementation of tsetse and trypanosomiasis eradication activities can contribute to all four pillars of CAADP. The elimination of T&T can liberate lands as well as ease access to water, it opens opportunities for agricultural and livestock activities as well as mixed farming in areas previously affected by T&T, provides animal traction to ease access to markets and facilitates agricultural research.

   The Meeting urged Governments of T&T affected countries to include the PATTEC Initiative in their respective national CAADP compact / programmes.

2. The Meeting noted that despite the efforts to remind affected countries of their obligations and of the advocacy carried out by the PATTEC Coordination Unit and various PATTEC stakeholders, many countries do not have a Tsetse and Trypanosomiasis unit to implement the PATTEC Initiative.

   Recalling that AU Member States adopted a declaration in Lome, Togo in July 2000, the Meeting recommended that all countries should be requested to establish PATTEC National Coordination Offices/Units dealing with T&T, provide the necessary resources (human, financial, and material) for the running of the Office/Unit as well as funding for field activities to commence tsetse and trypanosomiasis eradication activities. The Meeting also urged the countries to show greater commitments to the implementation of the PATTEC Initiative.

3. Recognizing the importance of trained human resources in all development programmes and considering that the number of experts in the field of tsetse and trypanosomiasis is

currently low due to many factors (age, no new trained personnel, etc.), the Meeting emphasized the necessity to recruit new staff to carry out tsetse and trypanosomiasis activities.

- The Meeting recommended that the PATTEC Coordination Office should make the necessary efforts to revive or set up a new training course for personnel specially dedicated to tsetse and trypanosomiasis in order to reinforce the capacity of all affected countries. More emphasis should be put on the training of middle level personnel in order for PATTEC projects to achieve T&T eradication.

4. Referring to the panel discussion on standards, the Meeting urged the PATTEC Coordination Office to propose standards on vector control to support countries in taking the right decision when procuring goods and services. Further, the Meeting recommended that the PATTEC Coordination Office should start discussions with OIE to include African Animal Trypanosomosis in the list of important diseases of the OIE.
5. Based on the lessons learned from the implementation of Phase One of the African Development Bank funded "Multinational project for the creation Tsetse and Trypanosomiasis free areas in East and West Africa", the Meeting recommended that the AU-PATTEC Coordination Office and the African Development Bank organize a joint meeting to evaluate the project and draw a road map for the future implementation of the PATTEC Initiative for the benefit of the affected countries.
6. Noting the role and functions of the PATTEC Coordination Office, the Meeting commended the PATTEC Coordination Office for its efforts to bring all stakeholders on board so that the T&T problem has a global appeal and recommended the Office to maintain this spirit of collaboration; further, the Meeting thanked and commended the AU Commission for its sustained support to the PATTEC Coordination Office and the implementation of the PATTEC Initiative.
7. The Meeting thanked the Government and the people of Ghana for hosting the 10th PATTEC National Coordinators' Meeting and for the hospitality extended to all participants.

The meeting was officially closed following the presentation of the summary report and the recommendations to the participants by Dr Hassane H. Mahamat AU- PATTEC Coordinator, by Dr Abebe Haile Gabriel, Director of Department of Rural Economy and Agriculture (DREA), and Dr Mark-Hansen, Director of Veterinary Services of the Republic of Ghana, representing the Deputy Minister of Food and Agriculture in charge of Livestock.

Dr Abebe Haile Gabriel thanked the participants and reiterated the support of his Department and the AUC to the PATTEC Coordination Office.

For further information contact: Dr. Hassane H. Mahamat, AU-PATTEC Coordinator (hassanehm@africa-union.org).

NEW FACT SHEET FROM WORLD HEALTH ORGANIZATION

Human African trypanosomiasis (sleeping sickness)

Fact sheet N° 259, January 2012

Key facts

- Sleeping sickness occurs only in 36 sub-Saharan Africa countries where there are tsetse flies that can transmit the disease.
- The people most exposed to the tsetse fly and therefore to the disease are in rural populations dependent on agriculture, fishing, animal husbandry or hunting.
- *Trypanosoma brucei gambiense (T. b. g.)* accounts for 95 percent of reported cases of sleeping sickness.
- After continued control efforts, the number of cases reported in 2009 has dropped below 10 000 for first time in 50 years. This trend has been maintained in 2010 with 7 139 new cases reported.
- Diagnosis and treatment of the disease are complex and require specifically skilled staff.

Definition of the disease

Human African trypanosomiasis, also known as sleeping sickness, is a vector-borne parasitic disease. The parasites concerned are protozoa belonging to the *Trypanosoma* genus. They are transmitted to humans by tsetse fly (*Glossina* genus) bites which have acquired their infection from human beings or from animals harbouring the human pathogenic parasites.

Tsetse flies are found just in sub-Saharan Africa though only certain species transmit the disease. For reasons that are so far unexplained, there are many regions where tsetse flies are found, but sleeping sickness is not. Rural populations living in regions where transmission occurs and which depend on agriculture, fishing, animal husbandry or hunting are the most exposed to the tsetse fly and therefore to the disease. The disease develops in areas ranging from a single village to an entire region. Within an infected area, the intensity of the disease can vary from one village to the next.

Forms of human African trypanosomiasis

Human African trypanosomiasis takes two forms, depending on the parasite involved:

- *Trypanosoma brucei gambiense (T. b. g.)* is found in West and Central Africa. This form currently accounts for over 95 percent of reported cases of sleeping sickness and causes a chronic infection. A person can be infected for months or even years without major signs or symptoms of the disease. When symptoms emerge, the patient is often already in an advanced disease stage where the central nervous system is affected.
- *Trypanosoma brucei rhodesiense (T. b. r.)* is found in eastern and southern Africa. Nowadays, this form represents under 5 percent of reported cases and causes an acute infection. First signs and symptoms are observed a few months or weeks after infection. The disease develops rapidly and invades the central nervous system.

  Another form of trypanosomiasis occurs mainly in 21 Latin American countries. It is known as American trypanosomiasis or Chagas disease. The causal organism is a different species from those causing the African form of the disease.

Animal trypanosomiasis

Other parasite species and sub-species of the *Trypanosoma* genus are pathogenic to animals and cause animal trypanosomiasis in wild and domestic animal species. In cattle the disease is called "*nagana"*, a Zulu word meaning "to be depressed".

Animals can host the human pathogen parasites, especially *T. b. rhodesiense*; thus domestic and wild animals are an important parasite reservoir. Animals can also be infected with *T. b. gambiense* and act as a reservoir. However the precise epidemiological role of this reservoir is not yet well known. The disease in domestic animals, particularly cattle, is a major obstacle to the economic development of affected rural areas.

Major human epidemics

There have been several epidemics in Africa over the last century:

- one between 1896 and 1906, mostly in Uganda and the Congo Basin
- one in 1920 in a number of African countries and
- the most recent epidemic occurred in 1970.

The 1920 epidemic was controlled thanks to mobile teams which organized the screening of millions of people at risk. By the mid-1960s, the disease had almost disappeared. After this success, surveillance was relaxed, and the disease reappeared in several areas over the last 30 years. The efforts of WHO, national control programmes, bilateral cooperation and non-governmental organizations (NGOs) during the 1990s and the beginning of the 21st century stopped and reversed the upward trend of new cases.

Distribution of the disease

Sleeping sickness threatens millions of people in 36 countries in sub-Saharan Africa. Many of the affected populations live in remote areas with limited access to adequate health services, which hampers the surveillance and therefore the diagnosis and treatment of cases. In addition, displacement of populations, war and poverty are important factors leading to increased transmission and this alters the distribution of the disease due to weakened or non-existent health systems.

- In 1986, it was estimated that some 70 million people lived in areas where disease transmission could take place.
- In 1998, almost 40 000 cases were reported, but estimates were that 300 000 cases were undiagnosed and therefore untreated.
- During epidemic periods prevalence reached 50 percent in several villages in the Democratic Republic of Congo, Angola and Southern Sudan. Sleeping sickness was the first or second greatest cause of mortality in those communities, ahead of even HIV/AIDS.
- By 2005, surveillance was reinforced and the number of new cases reported on the continent was reduced; between 1998 and 2004 the number of both forms of the disease fell from 37 991 to 17 616. The estimated number of actual cases was between 50 000 and 70 000.
- In 2009, after continued control efforts, the number of cases reported has dropped below 10 000 (9 878) for first time in 50 years. This trend has been maintained in 2010 with 7 139 new cases reported. The estimated number of actual cases is currently 30 000.

In 2000 and 2001, WHO established public-private partnerships with Aventis Pharma (now Sanofi-Aventis) and Bayer HealthCare which enabled the creation of a WHO surveillance

team, providing support to endemic countries in their control activities and the supply of drugs free of charge for the treatment of patients.

The partnership was renewed in 2006 and recently in 2011. The success in curbing the number of sleeping sickness cases encouraged other private partners to sustain the WHO's initial effort towards the elimination of the disease as a public health problem.

Current situation in endemic countries

The prevalence of the disease differs from one country to another as well as in different parts of a single country.

- In the last 10 years, over 70 percent of reported cases occurred in the Democratic Republic of Congo (DRC).
- In 2010, only the DRC declared over 500 new cases per year.
- Angola, Central African Republic, Chad, Sudan and Uganda declared between 100 and 500 new cases per year.
- Countries such as, Cameroon, Congo, Côte d'Ivoire, Equatorial Guinea, Gabon, Guinea, Malawi, Nigeria, United Republic of Tanzania, Zambia and Zimbabwe are reporting fewer than 100 new cases per year.
- Countries like Benin, Botswana, Burkina Faso, Burundi, Ethiopia, Gambia, Ghana, Guinea Bissau, Kenya, Liberia, Mali, Mozambique, Namibia, Niger, Rwanda, Senegal, Sierra Leone, Swaziland and Togo have not reported any new cases for over a decade. Transmission of the disease seems to have stopped but there are still some areas where it is difficult to assess the exact situation because the unstable social circumstances and/or remote accessibility hinders surveillance and diagnostic activities.

Infection and symptoms

The disease is mostly transmitted through the bite of an infected tsetse fly but there are other ways in which people are infected with sleeping sickness.

- Mother-to-child infection: the trypanosome can cross the placenta and infect the foetus.
- Mechanical transmission through other blood sucking insects is possible. However, it is difficult to assess the epidemiological impact of transmission.
- Accidental infections have occurred in laboratories due to pricks from contaminated needles.

In the first stage, the trypanosomes multiply in subcutaneous tissues, blood and lymph. This is known as a haemolymphatic phase, which entails bouts of fever, headaches, joint pains and itching.

In the second stage the parasites cross the blood-brain barrier to infect the central nervous system. This is known as the neurological phase. In general this is when more obvious signs and symptoms of the disease appear: changes of behaviour, confusion, sensory disturbances and poor coordination. Disturbance of the sleep cycle, which gives the disease its name, is an important feature of the second stage of the disease. Without treatment, sleeping sickness is considered fatal.

Disease management: diagnosis

Disease management is made in three steps.

1. Screening for potential infection. This involves using serological tests (only available for *T. b. gambiense*) and checking for clinical signs - generally swollen cervical glands.

2. Diagnosing whether the parasite is present.
3. Staging to determine the state of disease progression. This entails examining cerebro-spinal fluid obtained by lumbar puncture and is used to determine the course of treatment.

Diagnosis must be made as early as possible and before the neurological stage in order to avoid complicated, difficult and risky treatment procedures.

The long, relatively asymptomatic first stage of *T. b. gambiense* sleeping sickness is one of the reasons why an exhaustive, active screening of the population at risk is required, in order to identify patients at an early stage and reduce transmission. Exhaustive screenings require a major investment in human and material resources. In Africa such resources are often scarce, particularly in remote areas where the disease is mostly found. As a result, many infected individuals may die before they can ever be diagnosed and treated.

Treatment

The type of treatment depends on the stage of the disease. The drugs used in the first stage of the disease are of lower toxicity and easier to administer. The earlier the disease is identified, the better the prospect of a cure.

Treatment success in the second stage depends on a drug that can cross the blood-brain barrier to reach the parasite. Such drugs are toxic and complicated to administer. Four drugs are registered for the treatment of sleeping sickness and provided free of charge to endemic countries.

First stage treatment:

- **Pentamidine:** discovered in 1941, used for the treatment of the first stage of *T. b. gambiense* sleeping sickness. Despite non-negligible undesirable effects, it is in general well tolerated by patients.
- **Suramin:** discovered in 1921, used for the treatment of the first stage of *T. b. rhodesiense*. It provokes certain undesirable effects, in the urinary tract and allergic reactions.

Second stage treatment:

- **Melarsoprol:** discovered in 1949, it is used in both forms of infection. It is derived from arsenic and has many undesirable side effects. The most dramatic is reactive encephalopathy (encephalopathic syndrome) which can be fatal (3 percent to 10 percent). An increase in resistance to the drug has been observed in several foci particularly in central Africa.
- **Eflornithine:** this molecule, less toxic than melarsoprol, was registered in 1990. It is only effective against *T. b. gambiense*. The regimen is strict and difficult to apply.
- A combination treatment of **nifurtimox and eflornithine** has been recently introduced (2009). It simplifies the use of eflornithine in monotherapy, but unfortunately it is not effective for *T. b. rhodesiense*. Nifurtimox is registered for the treatment of American trypanosomiasis but not for human African trypanosomiasis. Nevertheless, after safety and efficacy data provided by clinical trials, its use in combination with eflornithine has been accepted and included in the WHO List of Essential Medicine, and it is provided free of charge for this purpose by WHO.

WHO response

WHO provides support and technical assistance to national control programmes. An important part of the response is a WHO private partnership with Sanofi-Aventis (pentamidine, melarsoprol and eflornithine) and Bayer AG (suramin and nifurtimox) to

provide the drugs free of charge to endemic countries. A network has been established for donor countries, private foundations, NGOs, regional institutions, research centres and universities to participate in surveillance and control, and to undertake research projects to develop new drugs and diagnostic tools.

The objectives of the WHO Programme are to:

- strengthen and coordinate control measures and ensure field activities are sustained;
- strengthen existing surveillance systems;
- ensure accessibility to diagnostic and treatment;
- support the monitoring of treatment and drug resistance throughout the network;
- develop information database and epidemiological analysis of data;
- implement training activities;
- support operational research to improve treatment and diagnostic tools;
- promote collaboration with the Food and Agriculture Organization (FAO) in charge of animal trypanosomiasis and the International Atomic Energy Agency (IAEA) dealing with vector control through male flies made sterile by radiation. The three UN agencies along with the African Union have promoted the Programme Against African Trypanosomiasis (PAAT);
- co-ordinate and synergize vector control activities lead by the Pan African Tsetse and Trypanosomosis Eradication Campaign of the African Union.

For more information contact:

WHO Media centre, Telephone: +41 22 791 2222; E-mail: mediainquiries@who.int

FAO ACTIVITIES IN THE FIELD OF TSETSE AND TRYPANOSOMOSIS CONTROL

In general, FAO activities in the field of tsetse and trypanosomosis include:

Partnership building: with relevant UN (IAEA and WHO) and continental (AU-PATTEC, AU-IBAR) organizations or institutions under the PAAT initiative or through bilateral agreements. This is materialized by co-organizing meetings and workshops to promote common understanding of problems and coordinated planning and implementation of solutions. In this respect an MOU was signed in July to officialise the collaboration between FAO and PATTEC (see above).

Normative work: production of guidelines delivered through publication in international journals or in the PAAT scientific and technical series, accessible on the PAAT website. In this respect it is worth mentioning the progress made on the Atlas of Human African Trypanosomosis, a WHO initiative jointly developed with FAO, and on a more recent initiative to develop an Atlas of Tsetse and African Animal Trypanosomosis.

Operational activities: These include assistance to countries and organizations in project formulation and implementation as well as the organization of or participation in training events related to capacity building or reinforcement organized by other partners like PAATEC

Assistance in formulation of new projects

Two (2) Technical Cooperation projects (TCPs) related to tsetse and trypanosomosis control have been formulated for Mali and Burkina Faso. The Mali project has been funded for $339

000 while that for Burkina is under evaluation. Another project related to exchange of trypanotolerant livestock between Mali and Guinea as provider countries on one side, and Liberia and Sierra Leone as demanding countries on the other, is being formulated under the initiative of the FAO Subregional office for West Africa (SFW). Also under planning is the formulation of a TCP for the cotton area of Côte d'Ivoire. This project combined with those in Mali and Burkina Faso will help cover the main cotton belt of West Africa which was identified as a priority area for tsetse and trypanosomosis control.

Assistance to ongoing projects

The project entitled "Development of innovative site-specific integrated animal health packages for the rural poor" was designed to address animal health problems that constrain productivity and the safety of livestock products in sub-Saharan Africa. The project is testing animal health packages centred on the Livestock Protective Net Fence (LPNF) in different production systems in Kenya, Ghana and Burkina Faso. The focus of the project in Kenya is on smallholder zero-grazing dairy farms, while in Ghana the focus is on small-scale pig producers in high tsetse threat areas, where pigs represent an important means to alleviate poverty. In Burkina Faso, project activities focus on small ruminants in the peri-urban areas of Bobo Dioulasso. Preliminary results are very encouraging in all countries and in order to obtain greater pro-poor impact, it is envisaged to provide these packages to other countries in East and West Africa through the organization of three workshops in Kenya, Ghana and Burkina Faso respectively. These workshops will be attended by 36 participants coming from the seven beneficiary countries, Eritrea, Ethiopia, Burundi, Rwanda, Kenya, Ghana and Burkina Faso, as well as from Liberia, Sierra Leone, Nigeria, Mali, Côte d'Ivoire and Senegal. This project started in June 2009 and will be completed in December 2013. It is funded by an IFAD contribution of $1 600 000.

The project "Projet pilote d'Appui à la Prévention et à la Lutte contre la Trypanosomose Animale en Angola", with a budget of $ 288 000 started in May 2011 and will be completed in December 2012 after a six-month extension. The overall objective is to improve animal health in four (4) provinces of Angola, namely Bengo, Kwanza Norte, Kwanza Sul and Luanda. More specifically, the aim is to build capacity in the field of integrated trypanosomosis control at the central and community levels. In this respect the Animal Health Officer-PAAT based in Accra contributed to the training of 25 national veterinary services personnel in the techniques of animal trypanosomosis diagnosis and control. The same personnel benefitted from courses on data collection and management from Giuliano Cecchi, the FAO consultant on GIS and data management.

Assistance in Training

The Animal Health officer-PAAT based in Accra contributed to the first PATTEC training course held in Bobo Dioulasso, while the FAO consultant on GIS and data management gave training in support to national staff from South Africa, Kenya, Uganda, Ghana, Burkina Faso and Mozambique. In addition, assistance in the field of GIS and data management and analysis is routinely provided to various technical and scientific partners including PATTEC, Ghana, Sudan, Uganda, IAEA and CIRAD.

WORK SUPPORTED BY THE JOINT FAO/IAEA AND IAEA TECHNICAL CO-OPERATION PROGRAMMES

1. COORDINATED RESEARCH PROJECTS (CRPs)

Final Research Coordination Meeting of CRP on "Improving SIT for Tsetse Flies through Research on their Symbionts and Pathogens" 26-30 March 2012, Vienna, Austria

The fourth RCM was held at IAEA headquarters in Vienna. Twenty-two participants from fifteen countries attended the meeting together with two consultants from the USA and France, and four observers (from Austria, Kenya, Italy and USA). The first two days of the meeting were devoted to presentations. During the remainder of the meeting the participants discussed the major achievements and the recommendations in two working groups dealing respectively with tsetse pathogens and tsetse symbionts.

During the discussion it was concluded that the CRP had been very productive, with a much better understanding of the many aspects of the physiology of the tsetse fly, including its fecundity, that are influenced by the fitness of its symbiotic fauna. However, correlations and interactions between the presence of virus, disease symptoms and the occurrence of bacterial symbionts (*Wigglesworthia*, *Sodalis* and *Wolbachia*) need to be further explored. These will be addressed by the new CRP on "Enhancing Vector Refractoriness to Trypanosome Infection" which was announced in the previous Volume of TTI.

The group discussed and emphasised the adoption of virus management strategies which have been designed and partially validated to mitigate / manage the disease. These strategies are based on: (i) monitoring viral loads for colony quality control; (ii) blocking transmission using specific antibodies, specific oligopeptides and/or clean feeding practices; and (iii) applying the drug valacyclovir to inhibit virus replication. The group also recommended strategies designed to: (i) monitor prevalence and loads of tsetse symbionts and pathogens; (ii) augment current feeding regimens to improve tsetse fecundity; (iii) improve the application of SIT by harnessing tsetse symbionts to develop pathogen resistant fly lines and to introduce natural sterility.

The CRP has resulted in some important achievements which will help in improving tsetse mass-production for SIT programmes. These achievements include the following:

- Potential improvement of tsetse mass-rearing through dietary supplementation (yeast extract);
- Discovery of various *Sodalis* genotypes in natural populations;
- Discovery of the functional role of *Wolbachia* in inducing high cytoplasmic incompatibility (CI) and development of a mathematical model to use CI for paratransgenic application to derive desirable phenotypes;
- Identification and characterization of the causal agent (SGHV) of salivary gland hypertrophy and identification of its mode of transmission in tsetse colonies;
- Analysis of the prevalence and genetic diversity of salivary gland hypertrophy in natural tsetse populations;
- Development of strategies to manage the virus infection in tsetse mass-rearing facilities;
- Dissemination of these discoveries to endemic countries and interested parties in Member States through the RCMs and two workshops;
- Research being published in a special issue of the Journal of Invertebrate Pathology.

2. TECHNICAL COOPERATION PROJECTS

Creating a Tsetse-Free Zone in the Southern Rift Valley

IAEA Deputy Director General Daud Bin Mohamad travelled to Ethiopia for a high level meeting (HLM) with the Minister for Science and Technology, Ato Dessie Dalkie, and others on the STEP tsetse project from 24 to 29 April 2012. The HLM was also attended by Ato Shiferaw Shigute, President of the Southern Nations, Nationalities and Peoples Region (SNNPR), Ato Sani Redi, Director of the Agricultural Bureau of SNNPR, a representative of the Federal Assembly and representatives for other partner ministries and organizations. The HLM was preceded by a two-day international management advisory committee (IMAC) meeting that was also attended by an international expert on SIT, Aldo Malavasi. The HLM reviewed the report of the IMAC and endorsed and approved the recommendations of the IMAC after some minor changes.

The IMAC reported considerable progress in the STEP project towards the completion of the pre-operational phase by August 2012. The progress includes the introduction of an appropriate mechanism for project oversight, the revision of the managerial set-up and the implementation of recommended actions in several technical areas related to the mass-production of sterile male tsetse flies and to the field operations. Furthermore, weekly aerial releases of sterile *Glossina fuscipes fuscipes* male flies were initiated in early April 2012 over the Deme basin.

The progress made was recognised in recent hearings in the Ethiopian House of Representatives and was broadly conveyed to the Ethiopian public through TV, radio and newspaper articles. Both HE Ato Dessie for the Government of Ethiopia and Mr Daud for the IAEA committed to intensify their support to the project to ensure completion of the remaining pre-operational tasks.

Following the HLM, the delegation visited the Deme valley where they viewed the aerial releases of sterile tsetse flies in the northwest section of the project area. The participants were able to observe the aircraft releasing boxes of sterile flies over this basin. The delegation then proceeded to Arba Minch where they observed the treatment of livestock with pour-on insecticides and insecticide impregnated targets deployed in tsetse habitats for pre-release tsetse fly suppression.

The visible benefits of the STEP tsetse population suppression activities are already impressive when compared with the initiation of STEP, when livestock in the project area were rare and required continuous protection based on the injection of trypanocidal drugs. The project has transformed the lives of farming communities as a result of the availability of oxen to plough the land, donkeys to pull carts to transport agricultural products to market, and meat and milk to improve human nutrition, creating thriving farming communities with dramatically improved living conditions.

As the rains had just started many farmers were busy ploughing using yokes of oxen or delivering agricultural goods to market using donkey carts, both possible only because of the good level of tsetse suppression already achieved by the project over the past few years in the northern area of the project area. The project is poised to start expanding sterile tsetse fly releases to eradicate tsetse to reach a long term sustainable solution to the tsetse and trypanosomosis problem in the southern Rift Valley of Ethiopia.

Supporting the Operational Phase of Eliminating *Glossina palpalis gambiensis* from the Niayes Area of Senegal

This project aims to eradicate the tsetse fly *Glossina palpalis gambiensis* from the Niayes and is now ready to enter its operational phase. A feasibility study that included the collection of entomological, veterinary, socio-economic and environmental baseline data, a population genetics study, the development of handling and pupal transport methods, trial releases of sterile male flies, mating compatibility studies etc. has been completed and has ascertained the feasibility of creating a sustainable zone free of *G. p. gambienis* in the Niayes using an area-wide integrated pest management approach with a sterile insect technique (SIT) component.

A group of external tsetse experts visited the project in May 2012 and ascertained that all activities of the feasibility study and the pre-operational phase had been accomplished and that the IAEA can proceed to provide support to the operational phase of the project.

A fly suppression trial was carried out in Kayhar (northern part of the project area), where a total of 305 insecticide-impregnated Vavoua traps were deployed at an average density of 30 traps/km^2 of "wet" area (i.e. preferred habitat). The suppression trial was monitored regularly and only two wild flies have been sampled in the monitoring traps since Feb 2011. The trial has indicated that the insecticide impregnated traps as a suppression tool have worked very well in this area that had low initial fly densities. However, it also demonstrated that the *G. palpalis gambiensis* fly population cannot be eradicated with insecticide traps alone, and hence confirms the need for the SIT as a final eradication component (as was also demonstrated in the Sidéradougou area of Burkina Faso in the 1980s and more recently on the Loos Islands of Guinea).

Shipments of sterile male pupae from the "Centre International de Recherche-Développement sur L'Élevage en Zone Subhumide" (CIRDES) in Burkina Faso to Dakar, Senegal were initiated in 2010 and test adult sterile male releases have been conducted on a weekly basis alternatively in Diaksao Peuhl and in the Parc de Hann. From May 2011 to May 2012, a total of 54 565 and 55 818 sterile males were released in Diaksao Peuhl (during 34 release sessions) and Hann (during 38 release sessions) respectively, with average daily mortality rates after the release of 17 percent and 20 percent, respectively. Daily mortality was for both sites 3 percent higher when the flies were released in unfavourable habitats as compared with favourable habitats. Sterile to wild male ratios were higher in Diaksao Peuhl than in Hann but the competitiveness was better in Hann than in Diaksao Peuhl. The competitiveness of the sterile males was for both sites better in the rainy season as compared with the dry season. Overall, the performance of the sterile flies, shipped as chilled male pupae from Burkina Faso to Dakar and released in two different eco-systems in the target area was very acceptable.

The new release machine, developed for chilled aerial tsetse male releases by a Mexican company (Mubarqui), has been tested with fruit flies and screwworm flies in Mexico and seems to work fine. It now needs to be shipped to Dakar for trials with *G. p. gambiensis*. Thereafter, operational aerial releases will start in the Kayhar area.

Supporting the Use of the Sterile Insect Technique for Area-Wide Tsetse and Trypanosomosis Management in Africa (Phase II)

Regional Training Course on Standardised Collection and Processing of Tsetse Flies for Molecular Population Genetic and Morphometric Analyses

Twenty three participants from 13 tsetse and trypanosomosis affected African Member States attended this FAO/IAEA Regional Training Course. The course was held at the Kenya

Agricultural Research Institute - Trypanosomiasis Research Centre (KARI-TRC) in Nairobi, Kenya, 23 January to 3 February 2012.

The training course involved lectures, laboratory and field activities, exposing the participants to (a) using GPS instruments for geo-referenced data collection; (b) working with databases for storing geo-referenced information gathered from entomological monitoring; (c) collecting tsetse samples for genotyping analysis using morphometric and molecular methods; (d) using geometric and morphometric applications in support of tsetse population genetic analysis; (e) using polymerase chain reaction (PCR) and sequence data in genotyping tsetse symbionts and pathogens; (f) using the collected and processed information for decision making in area-wide integrated pest management (AW-IPM) campaigns against the tsetse and trypanosomosis problem, possibly involving a tsetse sterile insect technique (SIT) component.

Regional Training Course on Standardised Entomological Monitoring, Data Collection and GIS-Aided Processing as Needed for AW-IPM of the Tsetse and Trypanosomosis Problem

Thirty four participants from 17 tsetse and trypanosomosis affected African Member States attended this FAO/IAEA Regional Training Course. The course was held at the IAEA Collaborating Centre "Centre International de Recherche-Développement sur L'Élevage en Zone Subhumide" (CIRDES) in Bobo-Dioulasso, Burkina Faso, 6–24 February 2012.

The training course involved lectures, laboratory and field activities, exposing the participants to (a) using tsetse presence / absence risk prediction maps for planning of entomological surveillance; (b) the principles of stratified sampling of tsetse target populations, and designing and implementing routine entomological monitoring; (c) using GPS instruments for geo-referenced data collection; (d) working with data bases for storing geo-referenced information gathered from entomological monitoring; (e) processing entomological monitoring information stored in databases using GIS software; and (f) using the collected and processed information for decision making in area-wide integrated pest management (AW-IPM) campaigns against the tsetse and trypanosomosis problem, possibly involving a tsetse SIT component.

Inauguration of CIRDES as the first IAEA Collaborating Centre in Africa, 24 February 2012, Bobo-Dioulasso, Burkina Faso

The Centre International de Recherche-Développement sur l'Élevage en zone Sub-humide (CIRDES) in Bobo-Dioulasso, Burkina Faso was inaugurated on 24 February 2012 as an "*IAEA Collaborating Centre on The Use of Sterile Insect Technique for Area-wide Integrated Management of Tsetse Fly Populations"*.

The formal inauguration of the first IAEA Collaborating Centre in Africa took place in the presence of Dr Valentine Gnapi Yaoré, Director General of CIRDES, Mr Liang Qu, Director of the Joint FAO/IAEA Division and representatives from Burkina Faso and from neighbouring CIRDES and IAEA Member States.

The event coincided with the last day of the above-mentioned regional training course hosted from 6-24 February 2012 by CIRDES which was carried out in support of the Pan African Tsetse and Trypanosomosis Eradication Campaign (PATTEC), following close consultations with PATTEC, FAO, WHO and several national PATTEC coordinators.

FOUNDATION FOR INNOVATIVE NEW DIAGNOSTICS (FIND)

New Grant to FIND from the German Government

The German Federal Ministry of Education and Research (BMBF) recently decided to grant EUR 7.5 million to FIND. The grant will support the ongoing development of a novel platform molecular technology to diagnose a variety of diseases, including malaria, sleeping sickness, visceral leishmaniasis and Chagas disease. This new funding is part of a EUR 20 million disbursement, announced last December by the German BMBF, to three product development partnerships (PDPs) through 2015 to develop innovative tools and treatments for neglected tropical diseases.

The new grant will allow FIND to continue development of the LAMP (loop-mediated amplification) tests, which are based on a simple yet highly sensitive DNA amplification method that can be used to diagnose numerous diseases. LAMP is a molecular diagnostic platform that detects pathogen DNA from patient samples with very high specificity and sensitivity at constant temperatures. In addition, results can be detected by the naked eye, rather than with the complicated detection equipment required for more conventional methods. The technology has been designed to be robust enough to be used in the rural African settings where tropical diseases are most prevalent.

"Thanks to this important commitment from the German Government, FIND and its partners will be in a position to continue supporting projects and activities aimed at the eventual elimination of a number of tropical diseases," said Philippe Jacon, Chief Executive Officer, FIND. "By using this grant to accelerate the development of a multi-disease diagnostic platform, FIND is committed to rapidly and cost-effectively bringing to the field tools that can have a tangible impact on the lives of those affected by these diseases."

Over the last five years, FIND has rolled out five novel assays, three for the rapid diagnosis of tuberculosis and two platform tools for the diagnosis of various diseases, including TB, sleeping sickness and malaria. Along with its aim to bring simple, effective and affordable tests to the point of treatment, FIND is also committed to building on past investments by taking advantage of existing technologies to develop multi-disease platforms that can be used by health workers to detect several diseases with a single instrument.

Dr Helge Braun, Parliamentary State Secretary at the BMBF, remarked "We are committed to sustaining research and development in the field of infectious diseases, including neglected tropical diseases that affect a large number of people worldwide. The BMBF wants to address these global challenges by supporting collaborations between industry, science, product development partnerships like FIND, and national health programmes."

GLOBAL ALLIANCE FOR LIVESTOCK VETERINARY MEDICINES (GALVmed)

GALVmed has recently signed two new research agreements under its DFID-funded trypanosomosis project with one of Europe's top-ranked universities for life science research. The agreements are with the Drug Discovery Unit, part of the College of Life Sciences at Dundee University, Scotland. In 2011, the College was awarded a prestigious *Excellence in Impact* award by the UK Secretary of State for Universities and Science and is committed to translational research, including in the area of neglected tropical diseases of man and cattle. The first of these agreements covers research in the university's Drug Discovery Unit which aims to develop new drugs for the treatment and possible prevention of animal African trypanosomosis (AAT) in cattle. The novel drug discovery and development research is being

led by Kevin Read, who is head of drug metabolism and pharmacokinetics at the Unit. This research builds on promising leads developed during work undertaken on the closely related disease human African trypanosomosis (HAT) – also known as sleeping sickness. To be effective for treatment of HAT, drugs need to be able to pass through the blood-brain barrier, but this is not necessary for AAT. A number of drug candidates that were rejected from Dundee's HAT programme are therefore now being re-evaluated as potential drugs for cattle. Work in Dundee began in January 2012 and is scheduled for completion by August 2013. By then it is hoped that at least one candidate drug will have been identified which has activity against the three parasites that cause AAT (*Trypanosoma congolense, T. vivax* and *T. brucei brucei*) and also has good potential for development as a veterinary product for treatment of cattle. The other new agreement with the University of Dundee covers work led by Professor Mike Ferguson, who heads a team working on trypanosome biochemistry, including the development of appropriate low-cost, high-quality diagnostics. This group aims to develop a test that can be used by livestock owners and veterinarians in remote areas to detect disease causing trypanosomes in cattle and be able to distinguish active from past infections. The plan is to develop a prototype lateral flow test together with BBInternational, a specialist diagnostic company which has development and manufacturing facilities in Dundee.

SECTION B – ABSTRACTS

1. GENERAL (INCLUDING LAND USE)

16037. **Abdel Razek, A. A., Watcharakorn, A. & Castillo, M., 2011.** Parasitic diseases of the central nervous system. *Neuroimaging Clinics of North America*, **21** (4): 815-841.

Diagnostic Radiology Department, Mansoura Faculty of Medicine, Mansoura, Egypt. [arazek@mans.edu.eg].

This article reviews the characteristic imaging appearances of parasitic diseases of the central nervous system, including cysticercosis, toxoplasmosis, cystic echinococcosis, schistosomiasis, amebiasis, malaria, sparganosis, paragonimiasis, and American and African trypanosomiases. Routine precontrast and postcontrast MR imaging helps in localization, characterization, delineation of extension, and follow-up of the parasitic lesions. Moreover, recently developed tools, such as diffusion, perfusion, and MR spectroscopy, help to differentiate parasitic diseases of the central nervous system from simulating lesions. Combining imaging findings with geographic prevalence, clinical history, and serologic tests is required for diagnosis of parasitic diseases of the central nervous system.

16038. **Andrews, K. T., Haque, A. & Jones, M. K., 2012.** HDAC inhibitors in parasitic diseases. *Immunology & Cell Biology*, **90** (1): 66-77.

Eskitis Institute for Cell and Molecular Therapies, Griffith University, Nathan, Queensland, Australia. [k.andrews@griffith.edu.au].

Parasitic diseases cause significant global morbidity and mortality, particularly in underdeveloped regions of the world. Malaria alone causes ~800 000 deaths each year, with children and pregnant women being at highest risk. There is no licensed vaccine available for any human parasitic disease and drug resistance is compromising the efficacy of many

available anti-parasitic drugs. This is driving drug discovery research on new agents with novel modes of action. Histone deacetylase (HDAC) inhibitors are being investigated as drugs for a range of diseases, including cancers and infectious diseases such as HIV/AIDS, and several parasitic diseases. This review focuses on the current state of knowledge of HDAC inhibitors targeted to the major human parasitic diseases such as malaria, schistosomiasis, trypanosomiasis, toxoplasmosis and leishmaniasis. Insights are provided into the unique challenges that will need to be considered if HDAC inhibitors are to be progressed towards clinical development as potential new anti-parasitic drugs.

16039. **Bisser, S. & Courtioux, B., 2012.** Sleeping sickness: end of the epidemic outbreak? *Revue Neurologique (Paris)*, **168** (3): 230-238.

Inserm UMR 1094, neuroépidémiologie tropicale, CNRS FR 3503 GEIST, Faculté de pharmacie, Institut d'épidémiologie neurologique et de neurologie tropicale, Université Limoges, 87025 Limoges, France. [ient@unilim.fr].

Sleeping sickness or human African trypanosomiasis is a parasitic disease transmitted by tsetse flies and therefore confined to its habitat, the central part of the African continent. Two disease forms are linked to two different parasites: *T. b. gambiense* and *T. b. rhodesiense*. Current epidemiological data and precise and dynamic mapping of foci suggest a real decrease of the disease. Not all areas are under control and resurgence can still not be avoided from the remote areas where the disease is endemic. However, recent advances in knowledge in parasite genetics are giving hope of control. In 2009, for the first time in the last 50 years, less than 10 000 cases were declared to the World Health Organization. Clinical trials allowed revising some clinical concepts and linking them with parasite genetics: both disease forms can show variations from asymptomatic, chronic to acute and are linked to genetic differences in the host or the parasite. Parasitological diagnosis may be facilitated by the introduction of individual rapid tests and PCR-based field tests. Knowledge about the mechanisms of brain invasion and screening of inflammatory molecules allow new marker combinations for staging but they do not avoid lumbar puncture. Therapeutic options remain limited but there is hope to develop a new orally available drug in a near future.

16040. **Brun, R. & Blum, J., 2012.** Human African trypanosomiasis. *Infectious Disease Clinics of North America*, **26** (2): 261-273.

Department Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland. [Reto.brun@unibas.ch].

Human African trypanosomiasis (sleeping sickness) is caused by the unicellular parasite *Trypanosoma brucei* and transmitted by tsetse flies. It occurs exclusively in sub-Saharan Africa, usually in rural areas affected by civil conflicts and neglected health systems. Reported cases are fewer than 10 000/year, which classifies it as one of the most neglected tropical diseases. Because sleeping sickness is fatal if not treated, it has to be included in the differential diagnosis of every febrile traveller returning from a game park in East Africa. Elimination of the disease is considered feasible providing better tools for diagnosis and treatment can be made available.

16041. **Camp, D., Davis, R. A., Campitelli, M., Ebdon, J. & Quinn, R. J., 2012.** Drug-like properties: guiding principles for the design of natural product libraries. *Journal of Natural Products*, **75** (1): 72-81.

Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia. [r.quinn@griffith.edu.au].

While natural products or their derivatives and mimics have contributed around 50 percent of current drugs, there has been no approach allowing front-loading of chemical space compliant with lead- and drug-like properties. The importance of physicochemical properties of molecules in the development of orally bioavailable drugs has been recognized. Classical natural product drug discovery has only been able to undertake this analysis retrospectively after compounds are isolated and structures elucidated. The present approach addresses front-loading of both extracts and subsequent fractions with desired physicochemical properties prior to screening for drug discovery. The physicochemical profiles of natural products active against two neglected disease targets, malaria and African trypanosomiasis, are presented based on this strategy. This approach can ensure timely development of natural product leads at a hitherto unachievable rate.

16042. **Creek, D. J., Anderson, J., McConville, M. J. & Barrett, M. P., 2012.** Metabolomic analysis of trypanosomatid protozoa. *Molecular & Biochemical Parasitology*, **181** (2): 73-84.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK. [Michael.barrett@glasgow.ac.uk].

Metabolomics aims to measure all low molecular weight chemicals within a given system in a manner analogous to transcriptomics, proteomics and genomics. In this review we highlight metabolomics approaches that are currently being applied to the kinetoplastid parasites, *Trypanosoma brucei* and *Leishmania* spp. The use of untargeted metabolomics approaches, made possible through advances in mass spectrometry and informatics, and stable isotope labelling have increased our understanding of the metabolism in these organisms beyond the views established using classical biochemical approaches. Set within the context of metabolic networks, predicted using genome-wide reconstructions of metabolism, new hypotheses on how to target aspects of metabolism to design new drugs against these protozoa are emerging.

16043. **Fairlamb, A. H., 2012.** Infectious disease: genomics decodes drug action. *Nature*, **482** (7384): 167-169.

College of Life Sciences, University of Dundee, Dundee, UK. [a.h.fairlamb@dundee.ac.uk].

No abstract available.

16044. **Fidalgo, L. M. & Gille, L., 2011.** Mitochondria and trypanosomatids: targets and drugs. *Pharmaceutical Research*, **28** (11): 2758-2770.

Departamento de Parasitologia, Instituto de Medicina Tropical Pedro Kouri, Apartado Postal No. 601, Marianao 13, Ciudad Habana, Cuba. [monzote@ipk.sld.cu].

The family Trypanosomatidae is responsible for important infectious diseases in humans: sleeping sickness, Chagas disease and leishmaniasis. Currently, development of effective

vaccines against these parasites remains an unrealized goal, and clinical management is based on chemotherapeutics. Cost, toxicity and resistance problems of conventional drugs result in an urgent need to identify and develop new therapeutic alternatives. The sound understanding of parasite biology is key for identifying novel lead structures and new drug targets. This article reviews current knowledge about mitochondrial drug targets and existing drugs against *Trypanosoma* and *Leishmania.* In the past, several targets in trypanosomatid mitochondria (electron transport chain, kDNA and topoisomerases, tRNA import and fatty acid synthesis) have been identified. It has been suggested that inhibition of certain targets is involved in triggering apoptosis by impairment of mitochondrial membrane potential and/or production of reactive oxygen species. The inhibitory mechanism of approved drugs, such as pentamidine, nifurtimox, artemisinin and atovaquone, is described in parallel with others products from preclinical studies. In spite of the large amount of genetic information, the analysis of the phenotype of the trypanosomatid mitochondrion in different life stages will remain a useful tool to design new active compounds with selective toxicity against these parasites.

16045. **Geurts, N., Opdenakker, G. & Van den Steen, P. E., 2012.** Matrix metalloproteinases as therapeutic targets in protozoan parasitic infections. *Pharmacology & Therapeutics*, **133** (3): 257-279.

Laboratory of Immunobiology, Rega Institute for Medical Research, University of Leuven, Leuven, Minderbroedersstraat 10, B3000 Leuven, Belgium. [Philippe.VandenSteen@rega.kuleuven.be].

Matrix metalloproteinases (MMPs) are associated with processes of tissue remodelling and are expressed in all infections with protozoan parasites. We here report the status of MMP research in malaria, trypanosomiasis, leishmaniasis and toxoplasmosis. In all these infections, the balances between MMPs and endogenous MMP inhibitors are disturbed, mostly in favour of active proteolysis. When the infection is associated with leukocyte influx into specific organs, immunopathology and collateral tissue damage may occur. These pathologies include cerebral malaria, sleeping sickness (human African trypanosomiasis), Chagas disease (human American trypanosomiasis), leishmaniasis and toxoplasmic encephalitis in immune-compromised hosts. Destruction of the integrity of the blood-brain barrier (BBB) is a common denominator that may be executed by leukocytic MMPs under the control of host cytokines and chemokines as well as influenced by parasite products. Mechanisms by which parasite-derived products alter host expression of MMP and endogenous MMP inhibitors have only been described for hemozoin (Hz) in malaria. Hence, understanding these interactions in other parasitic infections remains an important challenge. Furthermore, the involved parasites are also known to produce their own metalloproteinases, and this forms an extra stimulus to investigate MMP inhibitory drugs as therapeutics. MMP inhibitors (MMPIs) may dampen collateral tissue damage, as is anecdotally reported for tetracyclines as MMP regulators in parasite infections.

16046. **Masocha, W. & Kristensson, K., 2012.** Passage of parasites across the blood-brain barrier. *Virulence*, **3** (2): 202-212.

Department of Applied Therapeutics, Faculty of Pharmacy, Kuwait University, Kuwait City, Kuwait.

The blood-brain barrier (BBB) is a structural and functional barrier that protects the central nervous system (CNS) from invasion by blood-borne pathogens including parasites. However, some intracellular and extracellular parasites can traverse the BBB during the course of

infection and cause neurological disturbances and/or damage which are at times fatal. The means by which parasites cross the BBB and how the immune system controls the parasites within the brain are still unclear. In this review we present the current understanding of the processes utilized by two human neuropathogenic parasites, *Trypanosoma brucei* spp and *Toxoplasma gondii*, to cross the BBB and the consequences of CNS invasion. We also describe briefly other parasites that can invade the brain and how they interact with or circumvent the BBB. The roles played by parasite-derived and host-derived molecules during parasitic and white blood cell invasion of the brain are discussed.

16047. **Matovu, E., Kazibwe, A. J., Mugasa, C. M., Ndungu, J. M. & Njiru, Z. K., 2012.** Towards point-of-care diagnostic and staging tools for human African trypanosomiasis. *Journal of Tropical Medicine*, **2012**: 340538.

School of Veterinary Medicine, Makerere University, P.O. Box 7062, Kampala, Uganda.

Human African trypanosomiasis is a debilitating disease prevalent in rural sub-Saharan Africa. Control of this disease almost exclusively relies on chemotherapy that should be driven by accurate diagnosis, given the unacceptable toxicity of the few available drugs. Unfortunately, the available diagnostics are characterised by low sensitivities due to the inherent low parasitaemia in natural infections. Demonstration of the trypanosomes in body fluids, which is a prerequisite before treatment, often follows complex algorithms. In this paper, we review the available diagnostics and explore recent advances towards development of novel point-of-care diagnostic tests.

16048. **Namangala, B., 2012.** Contribution of innate immune responses towards resistance to African trypanosome infections. *Scandinavian Journal of Immunology*, **75** (1): 5-15.

Department of Paraclinical Studies, School of Veterinary Medicine, The University of Zambia, Lusaka, Zambia. [b.namangala@unza.zm].

During the course of African trypanosomiasis, an intact monocytic cell system appears to be crucial for the initiation and maintenance of antitrypanosome responses and could be critical for the survival of trypanosome-infected host. Monocytic cells in turn require support from other components of the innate immunity as well as adaptive immunity for effective and sustained control of trypanosome infections. In this review, the contribution of specific components of the innate immune system towards resistance to African trypanosomes is discussed in the context of host survival and the ideas presented are expected to stimulate more debate and research on host innate mechanisms of defence against African trypanosomiasis.

16049. **Nicoll-Griffith, D. A., 2012.** Use of cysteine-reactive small molecules in drug discovery for trypanosomal disease. *Expert Opinion on Drug Discovery*, **7** (4): 353-366.

Infectious Diseases Franchise, Discovery and Pre-clinical Sciences, Merck and Co., Office K11-2047B, 2000 Galloping Hill Road, Kenilworth, NJ 07033, USA. [deborah_nicoll-griffith@merck.com].

The roles of cysteine protease (CP) enzymes in the biochemistry and infectivity of the three trypanosomal parasitic infections, Chagas' disease, leishmaniasis and human African

trypanosomiasis which have been elucidated over the last three decades are summarized. Inhibitors of these enzymes, which act through trapping the active site cysteine with an electrophilic warhead, hold huge potential as therapeutic agents but the promise of these has yet to be realized in clinical studies. The article addresses aspects that ought to be considered in order to develop orally active CP inhibitors that are safe and effective therapies for trypanosomiasis. This article reviews findings from CP research in the trypanosomal field and recent advances in developing cysteine protease inhibitors (CPIs) of human cathepsin K, a related enzyme. Considerations such as intra- and extracellular localization of the CPs, off-target activities against human cathepsin enzymes and potential pro-drug inhibitors are reviewed. A description of odanacatib, a cathepsin K inhibitor currently in late stage development, is made to illustrate the attributes of a clinically viable CPI. The emerging role of CPs in a wide array of parasitic diseases is highlighted with the vision that CP inhibitors could become the "beta-lactams" of anti-parasitic treatments in the coming decades. New CPI research will see the optimization of intra- and extracellular enzyme targeting, reduction of off-target activities and better understanding of pharmacokinetic-pharmacodynamic interactions which will all lead to compounds with much improved efficacy and viability as clinical therapies.

16050. **Oladiran, A. & Belosevic, M., 2012.** Immune evasion strategies of trypanosomes: a review. *Journal of Parasitology*, **98** (2): 284-292.

Department of Biological Sciences, University of Alberta, Edmonton, Alberta, Canada.

Trypanosomes are digenetic protozoans that infect domestic and wild animals, as well as humans. They cause important medical and veterinary diseases, making them a major public health concern. There are many species of trypanosomes that infect virtually all vertebrate taxa. They typically cycle between insect or leech vectors and vertebrate hosts, and they undergo biochemical and morphological changes in the process. Trypanosomes have received much attention in the last four decades because of the diseases they cause and their remarkable armamentarium of immune evasion mechanisms. The completed genome sequences of trypanosomes have revealed an extensive array of molecules that contribute to various immune evasion mechanisms. The different species interact uniquely with their vertebrate hosts with a wide range of evasion strategies and some of the most fascinating immune evasion mechanisms, including antigenic variation that was first described in the trypanosomes. This review focuses on the variety of strategies that these parasites have evolved to evade or modulate immunity of endothermic and ectothermic vertebrates.

16051. **Pal, C. & Bandyopadhyay, U., 2012.** Redox-active antiparasitic drugs. *Antioxidants & Redox Signaling*, **17** (4): 555-582.

Department of Infectious Diseases and Immunology, Indian Institute of Chemical Biology, Kolkata, India. [ubandyo_1964@yahoo.com].

Parasitic diseases affect hundreds of millions of people worldwide and represent major health problems. Treatment is becoming extremely difficult due to the emergence of drug resistance, the absence of effective vaccines, and the spread of insecticide-resistant vectors. Thus, identification of affordable and readily available drugs against resistant parasites is a global research thrust. Susceptibility of many parasites to oxidative stress is a well-known phenomenon. Therefore, generation of reactive oxygen species (ROS) or inhibition of endogenous antioxidant enzymes would be a novel therapeutic approach to develop

antiparasitic drugs. This article highlights the unique metabolic pathways along with redox enzymes of unicellular (*Plasmodium falciparum, Trypanosoma cruzi, Trypanosoma brucei, Leishmania donovani, Entamoeba histolytica*, and *Trichomonas vaginalis*) and multicellular parasites (*Schistosoma mansoni*), which could be utilized to promote ROS-mediated toxicity. Enzymes involved in various vital redox reactions could be potential targets for drug development, and therefore the identification of redox-active antiparasitic drugs along with their mode of action will help researchers around the world in designing novel drugs in the future.

16052. **Parikh, P. P., Zheng, J., Logan-Klumper, F., Stoeckert, C. J., Jr., Louis, C., Topalis, P., Protasio, A. V., Sheth, A. P., Carrington, M., Berriman, M. & Sahoo, S. S., 2012.** The ontology for parasite lifecycle (OPL): towards a consistent vocabulary of lifecycle stages in parasitic organisms. *Journal of Biomedical Semantics*, **3** (1): 5.

The Kno.e.sis Center, Department of Computer Science and Engineering, Wright State University, Dayton, OH, USA; Center for Bioinformatics, Department of Genetics, University of Pennsylvania, 1420 Blockley Hall, 423 Guardian Drive, Philadelphia, Pennsylvania 19104, USA; The Wellcome Trust Sanger Institute, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 ISA, UK; Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge CB2 1QW, UK; Institute of Molecular Biology and Biotechnology, Foundation for Research and Technology-Hellas, and Dept. of Biology, University of Crete, 700 13 Heraklion, Crete, Greece; and Division of Medical Informatics, School of Medicine, Case Western Reserve University, Cleveland, OH, USA. [priti@knoesis.org].

Genome sequencing of many eukaryotic pathogens and the volume of data being available on public resources have created a clear requirement for a consistent vocabulary to describe the range of developmental forms of parasites. Consistent labelling of experimental data and external data in databases and the literature is essential for integration, cross database comparison, and knowledge discovery. The primary objective of this work was to develop a dynamic and controlled vocabulary that can be used for various parasites. The paper describes the ontology for parasite lifecycle (OPL) and discusses its application in parasite research. The OPL is based on the basic formal ontology (BFO) and follows the rules set by the OBO Foundry consortium. The first version of the OPL models complex life cycle stage details of a range of parasites, such as *Trypanosoma sp., Leishmania* sp., *Plasmodium* sp., and *Schistosoma* sp. In addition, the ontology also models necessary contextual details, such as host information, vector information, and anatomical locations. OPL is primarily designed to serve as a reference ontology for parasite life cycle stages that can be used for database annotation purposes and in the lab for data integration or information retrieval as exemplified in the application section. OPL is freely available and has been submitted to the BioPortal site of NCBO for public use and to the OBO Foundry. We believe that database and phenotype annotations using OPL will help run fundamental queries on databases to know more about gene functions and to find intervention targets for various parasites. The OPL is under continuous development and new parasites and/or terms are being added.

16053. **Rascalou, G., Pontier, D., Menu, F. & Gourbiere, S., 2012.** Emergence and prevalence of human vector-borne diseases in sink vector populations. *PLoS One*, 7 (5): e36858.

UMR 5244 CNRS-UPVD Ecologie et Evolution des Interactions, Université de

Perpignan Via Domitia, Perpignan, France. [gourbiere@univ-perp.fr].

Vector-borne diseases represent a major public health concern in most tropical and subtropical areas, and an emerging threat for more developed countries. Our understanding of the ecology, evolution and control of these diseases relies predominantly on theory and data on pathogen transmission in large self-sustaining "source" populations of vectors representative of highly endemic areas. However, there are numerous places where environmental conditions are less favourable to vector populations, but where immigration allows them to persist. We built an epidemiological model to investigate the dynamics of six major human vector borne-diseases in such non self-sustaining "sink" vector populations. The model was parameterized through a review of the literature, and we performed extensive sensitivity analysis to look at the emergence and prevalence of the pathogen that could be encountered in these populations. Despite the low vector abundance in typical sink populations, all six human diseases were able to spread in 15-55 percent of cases after accidental introduction. The rate of spread was much more strongly influenced by vector longevity, immigration and feeding rates, than by transmission and virulence of the pathogen. Prevalence in humans remained lower than 5 percent for dengue, leishmaniasis and Japanese encephalitis, but substantially higher for diseases with longer durations of infection - malaria and the American and African trypanosomiasis. Vector-related parameters were again the key factors, although their influence was lower than that on pathogen emergence. Our results emphasize the need for ecology and evolution to be considered in the context of metapopulations consisting of a mosaic of sink and source habitats, and to design vector control programmes not only targeting areas of high vector density, but working at a larger spatial scale.

16054. **Schmidt, T. J., Khalid, S. A., Romanha, A. J., Alves, T. M., Biavatti, M. W., Brun, R., Da Costa, F. B., de Castro, S. L., Ferreira, V. F., de Lacerda, M. V., Lago, J. H., Leon, L. L., Lopes, N. P., das Neves Amorim, R. C., Niehues, M., Ogungbe, I. V., Pohlit, A. M., Scotti, M. T., Setzer, W. N., de, N. C. S. M., Steindel, M. & Tempone, A. G., 2012.** The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases - part I. *Current Medicinal Chemistry*, **19** (14): 2128-2175.

Institute of Pharmaceutical Biology and Phytochemistry, University of Munster, Germany. [thomschm@uni-muenster.de].

Infections with protozoan parasites are a major cause of disease and mortality in many tropical countries of the world. Diseases caused by species of the genera *Trypanosoma* (human African trypanosomiasis and Chagas disease) and *Leishmania* (various forms of Leishmaniasis) are among the seventeen "Neglected Tropical Diseases" (NTDs) defined as such by WHO due to the neglect of financial investment into research and development of new drugs by a large part of pharmaceutical industry and neglect of public awareness in high income countries. Another major tropical protozoan disease is malaria (caused by various *Plasmodium* species), which - although not mentioned currently by the WHO as a neglected disease - still represents a major problem, especially to people living under poor circumstances in tropical countries. Malaria causes by far the highest number of deaths of all protozoan infections and is often (as in this review) included in the NTDs. These diseases threaten many millions of lives world-wide and they are mostly associated with poor socioeconomic and hygienic environment. Existing therapies suffer from various shortcomings, namely, a high degree of toxicity and unwanted effects, lack of availability and/or problematic application under the life conditions of affected populations. Development

of new, safe and affordable drugs is therefore an urgent need. Nature has provided an innumerable number of drugs for the treatment of many serious diseases. Among the natural sources for new bioactive chemicals, plants are still predominant. Their secondary metabolism yields an immeasurable wealth of chemical structures which has been and will continue to be a source of new drugs, directly in their native form and after optimization by synthetic medicinal chemistry. The current review, published in two parts, attempts to give an overview on the potential of such plant-derived natural products as antiprotozoal leads and/or drugs in the fight against NTDs.

16055. **Schmidt, T. J., Khalid, S. A., Romanha, A. J., Alves, T. M., Biavatti, M. W., Brun, R., Da Costa, F. B., de Castro, S. L., Ferreira, V. F., de Lacerda, M. V., Lago, J. H., Leon, L. L., Lopes, N. P., das Neves Amorim, R. C., Niehues, M., Ogungbe, I. V., Pohlit, A. M., Scotti, M. T., Setzer, W. N., de, N. C. S. M., Steindel, M. & Tempone, A. G., 2012.** The potential of secondary metabolites from plants as drugs or leads against protozoan neglected diseases - part II. *Current Medicinal Chemistry*, **19** (14): 2176-2228.

Institute of Pharmaceutical Biology and Phytochemistry, University of Munster, Germany. [thomschm@uni-muenster.de].

Infections with protozoan parasites are a major cause of disease and mortality in many tropical countries of the world. Diseases caused by species of the genera *Trypanosoma* (human African trypanosomiasis and Chagas disease) and *Leishmania* (various forms of leishmaniasis) are among the seventeen "Neglected Tropical Diseases" (NTDs) defined by the WHO. Furthermore, malaria (caused by various *Plasmodium* species) can be considered a neglected disease in certain countries and with regard to availability and affordability of the antimalarials. Living organisms, especially plants, provide an innumerable number of molecules with potential for the treatment of many serious diseases. The current review attempts to give an overview of the potential of such plant-derived natural products as antiprotozoal leads and/or drugs in the fight against NTDs. In part I, a general description was provided of the diseases, the current state of therapy and need for new therapeuticals, assay methods and strategies applied in the search for new plant derived natural products against these diseases and an overview on natural products of terpenoid origin with antiprotozoal potential. The present part II compiles the current knowledge of natural products with antiprotozoal activity that are derived from the shikimate pathway (lignans, coumarins, caffeic acid derivatives), quinones of various structural classes, compounds formed via the polyketide pathways (flavonoids and related compounds, chromenes and related benzopyrans and benzofurans, xanthones, acetogenins from *Annonaceae* and polyacetylenes) as well as the diverse classes of alkaloids. In total, both parts compile the literature on almost 900 different plant-derived natural products and their activity data, taken from over 800 references. These data, as the result of enormous efforts of numerous research groups world-wide, illustrate that plant secondary metabolites represent an immensely rich source of chemical diversity with an extremely high potential to yield a wealth of lead structures towards new therapies for NTDs. Only a small percentage of the roughly 200 000 plant species on earth has been studied chemically and only a small percentage of these plants or their constituents have been investigated for antiprotozoal activity. The repository of plant-derived natural products hence deserves to be investigated even more intensely than it has been up to present.

16056. **Seke Etet, P. F. & Mahomoodally, M. F., 2012.** New insights in staging and chemotherapy of African trypanosomiasis and possible contribution of medicinal plants. *Scientific World Journal*, **2012**: 343652.

Department of Neurological Sciences (DNNMMS), University of Verona, Via Delle Grazie 8, 37134 Verona, Italy.

Human African trypanosomiasis (HAT) is a fatal if untreated fly-borne neuroinflammatory disease caused by protozoa of the species *Trypanosoma brucei* (*T. b.*). The increasing trend of HAT cases has been reversed, but according to WHO experts, new epidemics of this disease could appear. In addition, HAT is still a considerable burden for the quality of life and economy in 36 sub-Saharan Africa countries with 15-20 million persons at risk. Following joint initiatives of WHO and private partners, the fight against HAT was strengthened, resulting in considerable breakthroughs. We present here what is currently known about the aetiology and pathogenesis of HAT and the new insights in the development of accurate tools and tests for disease staging and severity monitoring in the field. Also, we elaborate on the promising progress being made in the development of less toxic and more efficient trypanocidal drugs including the potential of medicinal plants and related alternative drug therapies.

16057. **Simarro, P. P., Franco, J. R., Cecchi, G., Paone, M., Diarra, A., Ruiz Postigo, J. A. & Jannin, J. G., 2012.** Human African trypanosomiasis in non-endemic countries (2000-2010). *Journal of Travel Medicine*, **19** (1): 44-53.

World Health Organization, Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, Geneva, Switzerland. [simarrop@who.int].

Human African trypanosomiasis (HAT) can affect travellers to sub-Saharan Africa, as well as migrants from disease endemic countries (DECs), posing diagnostic challenges to travel health services in non-disease endemic countries (non-DECs). Cases reported in journals have been collected through a bibliographic research and complemented by cases reported to the World Health Organization (WHO) during the process of obtaining anti-trypanosome drugs. These drugs are distributed to DECs solely by WHO. Drugs are also provided to non-DECs when an HAT case is diagnosed. However, in non-DECs pentamidine can also be purchased in the market due to its indication to treat Pneumocystis and *Leishmania* infections. Any request for drugs from non-DECs should be accompanied by epidemiological and clinical data on the patient. During the period 2000 - 2010, 94 cases of HAT were reported in 19 non-DECs. Seventy-two percent of them corresponded to the *Rhodesiense* form, whereas 28 percent corresponded to the *Gambiense* form. Cases of *Rhodesiense* HAT were mainly diagnosed in tourists after short visits to DECs, usually within a few days of return. The majority of them were in the first stage. Initial misdiagnosis with malaria or tick-borne diseases was frequent. Cases of *Gambiense* HAT were usually diagnosed several months after initial examination and subsequent to a variety of misdiagnoses. The majority were in the second stage. Patients affected were expatriates living in DECs for extended periods and refugees or economic migrants from DECs. It is concluded that the risk of HAT in travellers and migrants, albeit low, cannot be overlooked. In non-DECs, rarity, nonspecific symptoms, and lack of knowledge and awareness among health staff make diagnosis difficult. Misdiagnosis is frequent, thus leading to invasive diagnostic methods, unnecessary treatments, and increased risk of fatality. Centralized distribution of drugs for HAT by WHO enables an HAT surveillance system for non-DECs to be maintained. This system provides valuable information on disease transmission and complements data collected in DECs.

16058. **Singh, B., Fleury, C., Jalalvand, F. & Riesbeck, K., 2012.** Human pathogens utilize host extracellular matrix proteins laminin and collagen for adhesion and invasion of

the host. *FEMS Microbiology Reviews*. **E publication ahead of print, April 26.**

Medical Microbiology, Department of Laboratory Medicine Malmo, Skane University Hospital, Lund University, Malmo, Sweden. [kristian.riesbeck@med.lu.se].

Laminin (Ln) and collagen are multifunctional glycoproteins that play an important role in cellular morphogenesis, cell signalling, tissue repair and cell migration. These proteins are ubiquitously present in tissues as a part of the basement membrane (BM), constitute a protective layer around blood capillaries and are included in the extracellular matrix (ECM). As a component of BMs, both Lns and collagen(s), thus function as major mechanical containment molecules that protect tissues from pathogens. Invasive pathogens breach the basal lamina and degrade ECM proteins of interstitial spaces and connective tissues using various ECM-degrading proteases or surface-bound plasminogen and matrix metalloproteinases recruited from the host. Most pathogens associated with the respiratory, gastrointestinal, or urogenital tracts, as well as with the central nervous system or the skin, have the capacity to bind and degrade Lns and collagen(s) in order to adhere to and invade host tissues. In this review, we focus on the adaptability of various pathogens to utilize these ECM proteins as enhancers for adhesion to host tissues or as a target for degradation in order to breach the cellular barriers. The major pathogens discussed are *Streptococcus, Staphylococcus, Pseudomonas, Salmonella, Yersinia, Treponema, Mycobacterium, Clostridium, Listeria, Porphyromonas* and *Haemophilus*; *Candida, Aspergillus, Pneumocystis, Cryptococcus* and *Coccidioides; Acanthamoeba, Trypanosoma* and *Trichomonas*; retrovirus and papilloma virus.

16059. **Teixeira, S. M., de Paiva, R. M., Kangussu-Marcolino, M. M. & Darocha, W. D., 2012.** Trypanosomatid comparative genomics: contributions to the study of parasite biology and different parasitic diseases. *Genetics & Molecular Biology*, **35** (1): 1-17.

Departamento de Bioquimica e Imunologia, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil.

In 2005, draft sequences of the genomes of *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major*, also known as the Tri-Tryp genomes, were published. These protozoan parasites are the causative agents of three distinct insect-borne diseases, namely sleeping sickness, Chagas disease and leishmaniasis, all with a worldwide distribution. Despite the large estimated evolutionary distance among them, a conserved core of ~6 200 trypanosomatid genes was found among the Tri-Tryp genomes. Extensive analysis of these genomic sequences has greatly increased our understanding of the biology of these parasites and their host-parasite interactions. In this article, we review the recent advances in the comparative genomics of these three species. This analysis also includes data on additional sequences derived from other trypanosomatid species, as well as recent data on gene expression and functional genomics. In addition to facilitating the identification of key parasite molecules that may provide a better understanding of these complex diseases, genome studies offer a rich source of new information that can be used to define potential new drug targets and vaccine candidates for controlling these parasitic infections.

16060. **Traore, A., Alvarez, I., Fernandez, I., Perez-Pardal, L., Kabore, A., Ouedraogo-Sanou, G. M., Zare, Y., Tamboura, H. H. & Goyache, F., 2012.** Ascertaining gene flow patterns in livestock populations of developing countries: a case study of the Burkina Faso goat. *BMC Genetics*, **13** (1): 35.

INERA, 04 BP 8645 Ouagadougou 04, Burkina Faso; SERIDA-Deva, C/Camino de Rioseco 1225, E-33394 Gijón (Asturias), Spain; and Laboratoire National d'Elevage, 01 BP 7021 Ouagadougou 01, Burkina Faso. [traore_pa@yahoo.fr].

Introgression of Sahel livestock genes southwards in West Africa may be favoured by human activity and the increase of the duration of the dry seasons since the 1970's. The aim of this study is to assess the gene flow patterns in Burkina Faso goat and to ascertain the most likely factors influencing geographic patterns of genetic variation in the Burkina Faso goat population. A total of 520 goats were sampled in 23 different locations of Burkina Faso and genotyped for a set of 19 microsatellites (data deposited in the Dryad repository: http://dx.doi.org/10.5061/dryad.41h46j37). Although overall differentiation is poor (FST = 0.067 +/- 0.003), the goat population of Burkina Faso is far from being homogeneous. Barrier analysis pointed out the existence of: a) genetic discontinuities in the Central and Southeast Burkina Faso; and b) genetic differences within the goats sampled in the Sahel or the Sudan areas of Burkina Faso. Principal component analysis and admixture proportion scores were computed for each population sampled and used to construct interpolation maps. Furthermore, Population Graph analysis revealed that the Sahel and the Sudan environmental areas of Burkina Faso were connected through a significant number of extended edges, which would be consistent with the hypothesis of long-distance dispersal. Genetic variation of the Burkina Faso goat followed a geographic-related pattern. This pattern of variation is likely to be related to the presence of vectors of African animal trypanosomosis. The partial Mantel test identified the present Northern limit of trypanosome vectors as the most significant landscape boundary influencing the genetic variability of the Burkina Faso goat ($p = 0.008$). The contribution of Sahel goat genes to the goat populations in the northern and eastern parts of the Sudan-Sahel area of Burkina Faso was substantial. The presence of perennial streams explains the existence of trypanosome vectors. The southern half of the Nakambe river (Southern Ouagadougou) and the Mouhoun river loop determined, respectively, the Eastern and Northern limits for the expansion of Sahelian goat genes. Furthermore, results from partial Mantel test suggest that the introgression of Sahelian goat genes into Djallonke goat using human-influenced genetic corridors has a limited influence when compared with the biological boundary defined by the northern limits for the distribution of the tsetse fly. However, the genetic differences found between the goats sampled in Bobo Dioulasso and the other populations located in the Sudan area of Burkina Faso may be explained by the broad goat trade favoured by the main road of the country. The current analysis clearly suggests that genetic variation in Burkina Faso goats: a) follows a North to South cline; and b) is affected by the distribution of the tsetse fly that imposes a limit to Sahelian goat expansion due to their trypanosusceptibility. Here we show how extensive surveys on livestock populations can be useful to indirectly assess the consequences of climate change and human action in developing countries.

16061. **Welburn, S. C. & Maudlin, I., 2012.** Priorities for the elimination of sleeping sickness. *Advances in Parasitology*, **79**: 299-337.

Division of Pathway Medicine and Centre for Infectious Diseases, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Edinburgh, UK. [sue.welburn@ed.ac.uk].

.

Sleeping sickness describes two diseases, both fatal if left untreated: (i) Gambian sleeping sickness caused by *Trypanosoma brucei gambiense*, a chronic disease with average infection lasting around three years, and (ii) Rhodesian sleeping sickness caused by *T. b. rhodesiense*, an acute disease with death occurring within weeks of infection. Control of Gambian sleeping

sickness is based on case detection and treatment involving serological screening, followed by diagnostic confirmation and staging. In stage I, patients can remain asymptomatic as trypanosomes multiply in tissues and body fluids; in stage II, trypanosomes cross the blood-brain barrier, enter the central nervous system and, if left untreated, death follows. Staging is crucial as it defines the treatment that is prescribed; for both forms of disease, stage II involves the use of the highly toxic drug melarsoprol or, in the case of Gambian sleeping sickness, the use of complex and very expensive drug regimes. Case detection of *T. b. gambiense* sleeping sickness is known to be inefficient but could be improved by the identification of parasites using molecular tools that are, as yet, rarely used in the field. Diagnostics are not such a problem in relation to *T. b. rhodesiense* sleeping sickness, but the high level of under-reporting of this disease suggests that current strategies, reliant on self-reporting, are inefficient. Sleeping sickness is one of the "neglected tropical diseases" that attracts little attention from donors or policymakers. Proper quantification of the burden of sleeping sickness matters, as the primary reason for its 'neglect' is that the true impact of the disease is unknown, largely as a result of under-reporting. Certainly, elimination will not be achieved without vast improvements in field diagnostics for both forms of sleeping sickness especially if there is a hidden reservoir of "chronic carriers". Mass screening would be a desirable aim for *Gambiense* sleeping sickness and could be handled on a national scale in the endemic countries - perhaps by piggybacking on programmes committed to other diseases. As well as improved diagnostics, the search for non-toxic drugs for stage II treatment should remain a research priority. There is good evidence that thorough active case finding is sufficient to control *T. b. gambiense* sleeping sickness, as there is no significant animal reservoir. *Trypanosoma brucei rhodesiense* sleeping sickness is a zoonosis and control involves interrupting the fly-animal-human cycle, so some form of tsetse control and chemotherapy of the animal reservoir must be involved. The restricted application of insecticide to cattle is the most promising, affordable and sustainable technique to have emerged for tsetse control. Animal health providers can aid disease control by treating cattle and, when allied with innovative methods of funding (e.g. public-private partnerships) not reliant on the public purse, this approach may prove more sustainable. Sleeping sickness incidence for the 36 endemic countries has shown a steady decline in recent years and we should take advantage of the apparent lull in incidence and aim for elimination. This is feasible in some sleeping sickness foci but must be planned and paid for increasingly by the endemic countries themselves. The control and elimination of *T. b. gambiense* sleeping sickness may be seen as a public good as appropriate strategies depend on local health services for surveillance and treatment, but public-private funding mechanisms should not be excluded. It is timely to take up the tools available and invest in new tools - including novel financial instruments - to eliminate this disease from Africa.

2. TSETSE BIOLOGY

(a) REARING OF TSETSE FLIES

16062. **Abd-Alla, A. M., Adun, H., Parker, A. G., Vreysen, M. J. & Bergoin, M., 2012.** The antiviral drug valacyclovir successfully suppresses salivary gland hypertrophy virus (SGHV) in laboratory colonies of *Glossina pallidipes*. *PLoS One*, **7** (6): e38417.

Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria. [a.m.m.abd-alla@iaea.org].

Many species of tsetse flies are infected with a virus that causes salivary gland hypertrophy (SGH) symptoms associated with a reduced fecundity and fertility. A high prevalence of SGH

has been correlated with the collapse of two laboratory colonies of *Glossina pallidipes* and colony maintenance problems in a mass rearing facility in Ethiopia. Mass-production of *G. pallidipes* is crucial for programmes of tsetse control including the sterile insect technique (SIT), and therefore requires a management strategy for this virus. Based on the homology of DNA polymerase between salivary gland hypertrophy virus and herpes viruses at the amino acid level, two antiviral drugs, valacyclovir and acyclovir, classically used against herpes viruses were selected and tested for their toxicity on tsetse flies and their impact on virus replication. While long term *per os* administration of acyclovir resulted in a significant reduction of productivity of the colonies, no negative effect was observed in colonies fed with valacyclovir-treated blood. Furthermore, treatment of a tsetse colony with valacyclovir for 83 weeks resulted in a significant reduction of viral loads and consequently suppression of SGH symptoms. The combination of initial selection of SGHV-negative flies by non-destructive PCR, a clean feeding system, and valacyclovir treatment resulted in a colony that was free of SGH syndromes in 33 weeks. This is the first report of the use of a drug to control a viral infection in an insect and of the demonstration that valacyclovir can be used to suppress SGH in colonies of *G. pallidipes*.

(b) TAXONOMY, ANATOMY, PHYSIOLOGY, BIOCHEMISTRY

16063. **Attardo, G. M., Benoit, J. B., Michalkova, V., Yang, G., Roller, L., Bohova, J., Takac, P. & Aksoy, S., 2012.** Analysis of lipolysis underlying lactation in the tsetse fly, *Glossina morsitans. Insect Biochemistry & Molecular Biology*, **42** (5): 360-370.

Yale School of Public Health, Yale University, New Haven, CT 06520, USA. [geoffrey.attardo@yale.edu].

Female tsetse flies undergo viviparous reproduction, generating one larva each gonotrophic cycle. Larval nourishment is provided by the mother in the form of milk secretions. The milk consists mostly of lipids during early larval development and shifts to a balanced combination of protein and lipids in the late larval instars. Provisioning of adequate lipids to the accessory gland is an indispensable process for tsetse fecundity. This work investigates the roles of Brummer lipase (Bmm) and the adipokinetic hormone (AKH)/adipokinetic hormone receptor (AKHR) systems on lipid metabolism and mobilization during lactation in tsetse. The contributions of each system were investigated by a knockdown approach utilizing siRNA injections. Starvation experiments revealed that silencing of either system results in prolonged female lifespan. Simultaneous suppression of bmm and akhr prolonged survival further than either individual knockdown. Knockdown of akhr and bmm transcript levels resulted in high levels of whole body lipids at death, indicating an inability to utilize lipid reserves during starvation. Silencing of bmm resulted in delayed oocyte development. Respective reductions in fecundity of 20 percent and 50 percent were observed upon knockdown of akhr and bmm, while simultaneous knockdown of both genes resulted in 80 percent reduction of larval production. Omission of one bloodmeal during larvigenesis (nutritional stress) after simultaneous knockdown led to almost complete suppression of larval production. This phenotype likely results from tsetse's inability to utilize lipid reserves as loss of both lipolysis systems leads to accumulation and retention of stored lipids during pregnancy. This shows that both Bmm lipolysis and AKH/AKHR signalling are critical for lipolysis required for milk production during tsetse pregnancy, and identifies the underlying mechanisms of lipid metabolism critical to tsetse lactation. The similarities in the lipid metabolic pathways and other aspects of milk production between tsetse and mammals indicate that this fly could be used as a novel model for lactation research.

16064. **Benoit, J. B., Attardo, G. M., Michalkova, V., Takac, P., Bohova, J. & Aksoy, S., 2012.** Sphingomyelinase activity in mother's milk is essential for juvenile development: a case from lactating tsetse flies. *Biology of Reproduction,* **18**(17): 1-10.

Division of Epidemiology and Public Health, Yale School of Public Health, Yale University, New Haven, Connecticut, USA; and Section of Molecular and Applied Zoology, Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia. [joshua.benoit@yale.edu].

Sphingosine is a structural component of sphingolipids. The metabolism of phosphoethanolamine ceramide (sphingomyelin) by sphingomyelinase (SMase), followed by the breakdown of ceramide by ceramidase (CDase) yields sphingosine. Female tsetse fly is viviparous and generates a single progeny within her uterus during each gonotrophic cycle. The mother provides her offspring with nutrients required for development solely via intrauterine lactation. Quantitative PCR showed that acid smase1 (*asmase1*) increases in mother's milk gland during lactation. aSMase1 was detected in the milk gland and larval gut, indicating this protein is generated during lactation and consumed by the larva. The higher levels of SMase activity in larval gut contents indicate that this enzyme is activated by the low gut pH. In addition *cdase* is expressed at high levels in the larval gut. Breakdown of the resulting ceramide is likely accomplished by the larval gut secreted CDase, which allows absorption of sphingosine. We used the tsetse system to understand the critical role(s) of SMase and CDase during pregnancy and lactation and their downstream effects on adult progeny fitness. Reduction of *asmase1* by siRNA negatively impacted pregnancy and progeny performance, resulting in a 4-5 day extension in pregnancy, 10-15 percent reduction in pupal mass, lower pupal hatch rates, and impaired heat tolerance, reduced symbiont levels and fecundity of adult progeny. This study suggests that the SMase activity associated with tsetse lactation and larval digestion is similar in function to that of mammalian lactation and represents a critical process for juvenile development with important effects on the health of progeny during their adulthood.

16065. **Bringaud, F., Barrett, M. P. & Zilberstein, D., 2012.** Multiple roles of proline transport and metabolism in trypanosomatids. *Frontiers in Bioscience*, **17**: 349-374.

Centre de Résonance Magnétique des Systèmes Biologiques, UMR 5536, Université Bordeaux Segalen, CNRS, Bordeaux, France. [bringaud@rmsb.u-bordeaux].

Trypanosomatids are a large family of unicellular eukaryotes, many of which are parasites in higher eukaryotes including man. Much of our understanding of metabolism in these organisms has been gained from the study of the human infective representatives (*Trypanosoma brucei* subspecies, *Trypanosoma cruzi* and *Leishmania* spp.) which are transmitted by blood-feeding arthropods. The insect vectors of these parasites use proline as a principal carbon and energy source circulating in their haemolymph. Accordingly the insect-forms of the human infectious parasites have evolved to exploit abundant proline when in this environment, but being able to activate different biochemical pathways when in other environments. Interestingly, if glucose is available, metabolic capability can shift to make this carbohydrate the preferred substrate. Proline has also been shown to play key roles in osmoregulation, differentiation in representatives of the group and may even play a role in immunosuppression elicited by the American trypanosome *T. cruz*i. This review focuses on recent progress in understanding the different aspects of proline metabolism in trypanosomatids, with a particular interest on the insect forms.

16066. **Guerra, L., Stoffolano, J. G., Jr., Gambellini, G., Masci, V. L., Belardinelli, M. C. & Fausto, A. M., 2012.** Ultrastructure of the salivary glands of non-infected and infected glands in *Glossina pallidipes* by the salivary glands hypertrophy virus. *Journal of Invertebrate Pathology*. **Available online 18 April.**

Dipartimento per le Innovazioni dei sistemi Biologici, Agroalimentari e Forestali, Universita della Tuscia, Largo dell'Universita, 01100 Viterbo, Italy. [lauraguerra@unitus.it].

Light, scanning electron, and transmission electron microscopy analyses were conducted to examine the morphology and ultrastructure of the salivary glands of *Glossina pallidipes*. Three distinct regions, each with a characteristic composition and organization of tissues and cells, were identified: secretory, reabsorptive and proximal. When infected with the salivary gland hypertrophy (SGH) virus, glands showed a severe hypertrophy, accompanied by profound changes in their morphology and ultrastructure. In addition, the muscular fibres surrounding the secretory region of the glands were disrupted. The morphological alterations in the muscular tissue, caused by viral infection, could be an important aspect of the pathology and may shed light on the mode of action of the SGH virus. Results are discussed with regard to the potential effect of viral infection on normal salivation and on the ability of infected tsetse flies to transmit a trypanosome parasite.

16067. **Kariithi, H. M., Ince, I. A., Boeren, S., Abd-Alla, A. M., Parker, A. G., Aksoy, S., Vlak, J. M. & Oers, M. M., 2011.** The salivary secretome of the tsetse fly *Glossina pallidipes* (Diptera: Glossinidae) infected by salivary gland hypertrophy virus. *PLoS Neglected Tropical Diseases*, **5** (11): e1371.

Laboratory of Virology, Wageningen University, Wageningen, The Netherlands. [just.vlak@wur.nl].

The competence of the tsetse fly *Glossina pallidipes* (Diptera: Glossinidae) to acquire salivary gland hypertrophy virus (SGHV), to support virus replication and successfully transmit the virus depends on complex interactions between *Glossina* and SGHV macromolecules. Critical requisites to SGHV transmission are its replication and secretion of mature virions into the fly's salivary gland (SG) lumen. However, secretion of host proteins is of equal importance for successful transmission and requires cataloguing of *G. pallidipes* secretome proteins from hypertrophied and non-hypertrophied SGs. After electrophoretic profiling and in-gel trypsin digestion, saliva proteins were analysed by nano-LC-MS/MS. MaxQuant/Andromeda search of the MS data against the non-redundant (nr) GenBank database and a *G. morsitans morsitans* SG EST database, yielded a total of 521 hits, 31 of which were SGHV-encoded. On a false discovery rate limit of 1 percent and detection threshold of least two unique peptides per protein, the analysis resulted in 292 *Glossina* and 25 SGHV MS-supported proteins. When annotated by the Blast2GO suite, at least one gene ontology (GO) term could be assigned to 89.9 percent (285/317) of the detected proteins. Five (approximately 1.8 percent) *Glossina* and three (approximately 12 percent) SGHV proteins remained without a predicted function after blast searches against the nr database. Sixty-five of the 292 detected *Glossina* proteins contained an N-terminal signal/secretion peptide sequence. Eight of the SGHV proteins were predicted to be non-structural (NS), and fourteen are known structural (VP) proteins. It is concluded that SGHV alters the protein expression pattern in *Glossina*. The *G. pallidipes* SG secretome encompasses a spectrum of proteins that may be required during the SGHV infection cycle. These detected proteins have putative interactions with at least 21 of the 25 SGHV-encoded proteins. Our findings open venues for

developing novel SGHV mitigation strategies to block SGHV infections in tsetse production facilities such as using SGHV-specific antibodies and phage display-selected gut epithelia-binding peptides.

16068. **Liu, R., He, X., Lehane, S., Lehane, M., Hertz-Fowler, C., Berriman, M., Field, L. M. & Zhou, J. J., 2012.** Expression of chemosensory proteins in the tsetse fly *Glossina morsitans morsitans* is related to female host-seeking behaviour. *Insect Molecular Biology*, **21** (1): 41-48.

Department of Biological Chemistry, Rothamsted Research, Harpenden, UK. [jiang.zhou@bbsrc.ac.uk].

Chemosensory proteins (CSPs) are a class of soluble proteins present in high concentrations in the sensilla of insect antennae. It has been proposed that they play an important role in insect olfaction by mediating interactions between odorants and odorant receptors. Here we report, for the first time, the presence of five CSP genes in the tsetse fly *Glossina morsitans morsitans*, a major vector transmitting nagana in livestock. Real-time quantitative reverse transcription PCR showed that three of the CSPs are expressed in antennae. One of them, GmmCSP2, is transcribed at a very high level and could be involved in olfaction. We also determined expression in the antennae of both males and females at different life stages and with different blood feeding regimes. The transcription of GmmCSP2 was lower in male antennae than in females, with a sharp increase in 10-week-old flies, 48 h after a bloodmeal. Thus there is a clear relationship between CSP gene transcription and host searching behaviour. Genome annotation and phylogenetic analyses comparing *G. morsitans morsitans* CSPs with those of other Diptera showed rapid evolution after speciation of mosquitoes.

16069. **Rotureau, B., Subota, I., Buisson, J. & Bastin, P., 2012.** A new asymmetric division contributes to the continuous production of infective trypanosomes in the tsetse fly. *Development*, **139** (10): 1842-1850.

Trypanosome Cell Biology Unit, Institut Pasteur & CNRS, URA 2581, 25 rue du Docteur Roux, 75015 Paris, France.

African trypanosomes are flagellated protozoan parasites that cause sleeping sickness and are transmitted by the bite of the tsetse fly. To complete their life cycle in the insect, trypanosomes reach the salivary glands and transform into the metacyclic infective form. The latter are expelled with the saliva at each blood meal during the whole life of the insect. Here, we reveal a means by which the continuous production of infective parasites could be ensured. Dividing trypanosomes present in the salivary glands of infected tsetse flies were monitored by live video-microscopy and by quantitative immunofluorescence analysis using molecular markers for the cytoskeleton and for surface antigens. This revealed the existence of two distinct modes of trypanosome proliferation occurring simultaneously in the salivary glands. The first cycle produces two equivalent cells that are not competent for infection and are attached to the epithelium. This mode of proliferation is predominant at the early steps of infection, ensuring a rapid colonization of the glands. The second mode is more frequent at later stages of infection and involves an asymmetric division. It produces a daughter cell that matures into the infective metacyclic form that is released in the saliva, as demonstrated by the expression of specific molecular markers - the calflagins. The levels of these calcium-binding proteins increase exclusively in the new flagellum during the asymmetric division, showing the commitment of the future daughter cell to differentiation. The coordination of

these two alternative cell cycles contributes to the continuous production of infective parasites, turning the tsetse fly into an efficient and long-lasting vector for African trypanosomes.

(c) DISTRIBUTION, ECOLOGY, BEHAVIOUR, POPULATION STUDIES

16070. **Balmand, S., Lohs, C., Aksoy, S. & Heddi, A., 2012.** Tissue distribution and transmission routes for the tsetse fly endosymbionts. *Journal of Invertebrate Pathology*. **Available online 18 April.**

INSA-Lyon, INRA, UMR203 BF2I, Biologie Fonctionnelle Insectes et Interactions, F-69621 Villeurbanne, France; Université de Lyon, F-69003 Lyon, France. [Severine.Balmand@jouy.inra.fr].

The tsetse fly *Glossina* is the vector of the protozoan *Trypanosoma brucei* spp., which causes Human and Animal African trypanosomiases in sub-Saharan African countries. To supplement their unbalanced vertebrate bloodmeal diet, flies permanently harbour the obligate bacterium *Wigglesworthia glossinidia*, which resides in bacteriocytes in the midgut bacteriome organ as well as in milk gland organ. Tsetse flies also harbour the secondary facultative endosymbionts (S-symbiont) *Sodalis glossinidius* that infects various tissues and *Wolbachia* that infects germ cells. Tsetse flies display viviparous reproductive biology where a single embryo hatches and completes its entire larval development *in utero* and receives nourishments in the form of milk secreted by mother's accessory glands (milk glands). To analyse the precise tissue distribution of the three endosymbiotic bacteria and to infer the way by which each symbiotic partner is transmitted from parent to progeny, we conducted a fluorescence *in situ* hybridization (FISH) study to survey bacterial spatial distribution across the fly tissues. We show that bacteriocytes are mono-infected with *Wigglesworthia*, while both *Wigglesworthia* and *Sodalis* are present in the milk gland lumen. *Sodalis* was further seen in the uterus, spermathecae, fat body, milk and intracellular spaces in the milk gland cells. Contrary to *Wigglesworthia* and *Sodalis*, *Wolbachia* were the only bacteria infecting oocytes, trophocytes, and embryos at early embryonic stages. Furthermore, *Wolbachia* were not seen in the milk gland and in the fat body. This work further highlights the diversity of symbiont interactions in multipartner associations and supports two maternal routes of symbiont inheritance in the tsetse fly: *Wolbachia* through oocytes, and *Wigglesworthia* and *Sodalis* by means of milk gland bacterial infection at early post-embryonic stages.

16071. **De Meeus, T., Ravel, S., Rayaisse, J. B., Courtin, F. & Solano, P., 2012.** Understanding local population genetics of tsetse: The case of an isolated population of *Glossina palpalis gambiensis* in Burkina Faso. *Infection Genetics & Evolution*, **12** (6): 1229-1234.

Institut de Recherche pour le Développement (IRD), Centre International de Recherche pour l'Elevage en zones Subhumides (CIRDES), IRD UMR INTERTRYP IRD-CIRAD, CIRDES 01 BP 454 Bobo-Dioulasso 01, Burkina Faso; and Institut de Recherche pour le Développement (IRD), UMR INTERTRYP IRD-CIRAD, Campus International de Baillarguet, 34398 Montpellier Cedex 5, France. [thierry.demeeus@ird.fr].

Tsetse flies are the vectors of human and animal trypanosomiases. For tsetse eradication programmes, it is crucial to be able to identify and target isolated populations, because they can be targeted for eradication without risk of reinvasion. However, most data that are

available on non-isolated populations fail to find how these populations are locally structured, because the Wahlund effect (admixture of individuals from genetically different units) always interferes with interpretations. In this paper, we investigated the genetic population structure of a possibly isolated population of *Glossina palpalis gambiensis* in a sacred wood in south Burkina Faso, using microsatellite DNA markers. We found that genotypic proportions in this population were in agreement with a random mating model and that these tsetse were genetically highly differentiated from other populations of the same Mouhoun river basin only a few kilometres away, confirming their genetic isolation. The population also displayed substantial temporal differentiation in a two-year period that lead to an estimate of effective population size of approximately 100 individuals. The fact that no Wahlund effect was identified allowed us to accurately measure the basic genetic parameters of this isolated population. Identifying such isolated and small populations is crucial for eradication programmes and should be implemented more often.

16072. **Doudoumis, V., Tsiamis, G., Wamwiri, F., Brelsfoard, C., Alam, U., Aksoy, E., Dalaperas, S., Abd-Alla, A., Ouma, J., Takac, P., Aksoy, S. & Bourtzis, K., 2012.** Detection and characterization of *Wolbachia* infections in laboratory and natural populations of different species of tsetse flies (genus *Glossina*). *BMC Microbiology*, **12 Suppl 1**: S3.

Department of Environmental and Natural Resources Management, University of Ioannina, 2 Seferi St, 30100 Agrinio, Greece. [kbourtz@uoi.gr].

Wolbachia is a genus of endosymbiotic alpha-Proteobacteria infecting a wide range of arthropods and filarial nematodes. *Wolbachia* is able to induce reproductive abnormalities such as cytoplasmic incompatibility (CI), thelytokous parthenogenesis, feminization and male killing, thus affecting the biology, ecology and evolution of its hosts. The bacterial group has prompted research regarding its potential for the control of agricultural and medical disease vectors, including *Glossina* spp., which transmits African trypanosomes, the causative agents of sleeping sickness in humans and nagana in animals. In the present study, we employed a *Wolbachia* specific 16S rRNA PCR assay to investigate the presence of *Wolbachia* in six different laboratory stocks as well as in natural populations of nine different *Glossina* species originating from 10 African countries. *Wolbachia* was prevalent in *Glossina morsitans morsitans*, *G. morsitans centralis* and *G. austeni* populations. It was also detected in *G. brevipalpis*, and, for the first time, in *G. pallidipes* and *G. palpalis gambiensis*. On the other hand, *Wolbachia* was not found in *G. p. palpalis, G. fuscipes fuscipes* and *G. tachinoides*. *Wolbachia* infections of different laboratory and natural populations of *Glossina* species were characterized using 16S rRNA, the wsp (*Wolbachia* surface protein) gene and MLST (multi locus sequence typing) gene markers. This analysis led to the detection of horizontal gene transfer events, in which *Wolbachia* genes were inserted into the tsetse fly nuclear genome. In conclusion, *Wolbachia* infections were detected in both laboratory and natural populations of several different *Glossina* species. The characterization of these *Wolbachia* strains promises to lead to a deeper insight into tsetse fly-*Wolbachia* interactions, which is essential for the development and use of *Wolbachia*-based biological control methods.

16073. **Kariithi, H. M., Ahmadi, M., Parker, A. G., Franz, G., Ros, V. I., Haq, I., Elashry, A. M., Vlak, J. M., Bergoin, M., Vreysen, M. J. & Abd-Alla, A. M., 2012.** Prevalence and genetic variation of salivary gland hypertrophy virus in wild populations of the tsetse fly *Glossina pallidipes* from southern and eastern Africa. *Journal of Invertebrate Pathology,* **109**: 134-142.

Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, IAEA, Vienna, Austria; Laboratory of Virology, Wageningen University, Wageningen, The Netherlands; Laboratoire de Pathologie Comparée, Université Montpellier 2, Montpellier, France; and National Agriculture Research Centre, Park Road, Islamabad, Pakistan. [henry.kariithi@wur.nl].

The *Glossina pallidipes* salivary gland hypertrophy virus (GpSGHV) is a rod-shaped, non-occluded double-stranded DNA virus that causes salivary gland hypertrophy (SGH) and reduced fecundity in the tsetse fly *G. pallidipes*. High GpSGHV prevalence (up to 80 percent) makes it impossible to mass-rear *G. pallidipes* colonies for the sterile insect technique (SIT). To evaluate the feasibility of molecular-based GpSGHV management strategies, we investigated the prevalence and genetic diversity of GpSGHV in wild populations of *G. pallidipes* collected from ten geographical locations in eastern and southern Africa. Virus diversity was examined using a total sequence of 1 497 nucleotides (approximately 1 percent of the GpSGHV genome) from five putative conserved ORFs, p74, pif1, pif2, pif3 and dnapol. Overall, 34.08 percent of the analysed flies (n=1 972) tested positive by nested PCR. GpSGHV prevalence varied from 2 percent to 100 percent from one location to another but phylogenetic and gene genealogy analyses using concatenated sequences of the five putative ORFs revealed low virus diversity. Although no correlation of the virus diversity to geographical locations was detected, the GpSGHV haplotypes could be assigned to one of two distinct clades. The reference (Tororo) haplotype was the most widely distributed, and was shared by 47 individuals in seven of the 11 locations. The Ethiopian haplotypes were restricted to one clade, and showed the highest divergence (with 14-16 single nucleotide mutation steps) from the reference haplotype. The current study suggests that the proposed molecular-based virus management strategies have a good prospect of working throughout eastern and southern Africa due to the low diversity of the GpSGHV strains.

16074. **Mediannikov, O., Audoly, G., Diatta, G., Trape, J. F. & Raoult, D., 2012.** New *Rickettsia* sp. in tsetse flies from Senegal. *Comparative Immunology, Microbiology & Infectious Diseases*, **35** (2): 145-150.

Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes: Université de la Méditerranée, Marseille, France; and Institut de Recherche pour le Développement (IRD), Campus commun UCAD-IRD of Hann, URMITE UMR 198, BP 1386 CP 18524 Dakar, Senegal [didier.raoult@gmail.com].

Tsetse flies are blood-sucking insects transmitting African trypanosomiasis. They are known to harbour also three intracellular bacteria that play important role in their lifecycle: *Wigglesworthia glossinidia*, *Sodalis glossinidius* and *Wolbachia* sp. We have studied 78 *Glossina morsitans submorsitans* collected in Senegal. In all studied flies we amplified genes of the bacterium phylogenetically close to the obligate intracellular pathogen *Rickettsia felis*, the agent of spotted fever in humans. We also visualized this rickettsia in the cells of tsetse flies by fluorescence *in situ* hybridization. The role of this probable fourth endosymbiotic bacterium of tsetse flies in *Glossina* lifecycle and possible pathogenicity for humans should be further investigated.

16075. **Rio, R. V., Symula, R. E., Wang, J., Lohs, C., Wu, Y. N., Snyder, A. K., Bjornson, R. D., Oshima, K., Biehl, B. S., Perna, N. T., Hattori, M. & Aksoy, S., 2012.** Insight into the transmission biology and species-specific functional capabilities of tsetse (Diptera: Glossinidae) obligate symbiont *Wigglesworthia*. *MBio*, **3** (1): e00240-11

Department of Biology, West Virginia University, Morgantown, West Virginia, USA. [serap.aksoy@yale.edu].

Ancient endosymbionts have been associated with extreme genome structural stability with little differentiation in gene inventory between sister species. Tsetse flies (Diptera: Glossinidae) harbour an obligate endosymbiont, *Wigglesworthia*, which has coevolved with the *Glossina* radiation. We report on the ~720-kb *Wigglesworthia* genome and its associated plasmid from *Glossina morsitans morsitans* and compare them to those of the symbiont from *Glossina brevipalpis*. While there was overall high synteny between the two genomes, a large inversion was noted. Furthermore, symbiont transcriptional analyses demonstrated host tissue and development-specific gene expression supporting robust transcriptional regulation in *Wigglesworthia*, an unprecedented observation in other obligate mutualist endosymbionts. Expression and immunohistochemistry confirmed the role of flagella during the vertical transmission process from mother to intrauterine progeny. The expression of nutrient provisioning genes (thiC and hemH) suggests that *Wigglesworthia* may function in dietary supplementation tailored toward host development. Furthermore, despite extensive conservation, unique genes were identified within both symbiont genomes that may result in distinct metabolomes impacting host physiology. One of these differences involves the chorismate, phenylalanine, and folate biosynthetic pathways, which are uniquely present in *Wigglesworthia morsitans*. Interestingly, African trypanosomes are auxotrophs for phenylalanine and folate and salvage both exogenously. It is possible that *W. morsitans* contributes to the higher parasite susceptibility of its host species. Genomic stasis has historically been associated with obligate endosymbionts and their sister species. Here we characterize the *Wigglesworthia* genome of the tsetse fly species *Glossina morsitans* and compare it to its sister genome within *G. brevipalpis*. The similarity and variation between the genomes enabled specific hypotheses regarding functional biology. Expression analyses indicate significant levels of transcriptional regulation and support development- and tissue-specific functional roles for the symbiosis previously not observed in obligate mutualist symbionts. Retention of the genetically expensive flagella within these small genomes was demonstrated to be significant in symbiont transmission and tailored to the unique tsetse fly reproductive biology. Distinctions in metabolomes were also observed. We speculate an additional role for *Wigglesworthia* symbiosis where infections with pathogenic trypanosomes may depend upon symbiont species-specific metabolic products and thus influence the vector competence traits of different tsetse fly host species.

16076. **Schneider, D. I., Garschall, K. I., Parker, A. G., Abd-Alla, A. M. & Miller, W. J., 2012.** Global *Wolbachia* prevalence, titre fluctuations and their potential of causing cytoplasmic incompatibilities in tsetse flies and hybrids of *Glossina morsitans* subgroup species. *Journal of Invertebrate Pathology*. **Available online 10 April.**

Laboratories of Genome Dynamics, Department Cell and Developmental Biology, Center of Anatomy and Cell Biology, Medical University of Vienna, Vienna, Austria; and Insect Pest Control Laboratory, Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture, Vienna, Austria. [wolfgang.miller@meduniwien.ac.at]

We demonstrate the high applicability of a novel VNTR-based (variable-number-tandem-repeat) molecular screening tool for fingerprinting *Wolbachia*-infections in tsetse flies. The VNTR-141 locus provides reliable and concise differentiation between *Wolbachia* strains deriving from *Glossina morsitans morsitans*, *Glossina morsitans centralis*, and *Glossina brevipalpis*. Moreover, we show that certain *Wolbachia* infections in *Glossina* spp. are

capable of escaping standard PCR screening methods by "hiding" as low-titre infections below the detection threshold. By applying a highly sensitive PCR-blot technique to our *Glossina* specimen, we were able to enhance the symbiont detection limit substantially and, consequently, trace unequivocally *Wolbachia* infections at high prevalence in laboratory-reared *G. swynnertoni* individuals. To our knowledge, *Wolbachia* persistence was reported exclusively for field-collected samples, and at low prevalence only. Finally, we highlight the substantially higher *Wolbachia* titre levels found in hybrid *Glossina* compared to non-hybrid hosts and the possible impact of these titres on hybrid host fitness that potentially triggers incipient speciation in tsetse flies.

16077. **Wang, J. & Aksoy, S., 2012.** PGRP-LB is a maternally transmitted immune milk protein that influences symbiosis and parasitism in tsetse's offspring. *Proceedings of the National Academy of Sciences U S A*, **109** (26): 10552-10557.

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

Beneficial microbe functions range from host dietary supplementation to development and maintenance of host immune system. In mammals, new-born progeny are quickly colonized with a symbiotic fauna that is provisioned in mother's milk and that closely resembles that of the parent. Tsetse fly (Diptera: Glossinidae) also depends on the obligate symbiont *Wigglesworthia* for nutritional supplementation, optimal fecundity, and immune system development. Tsetse progeny develop one at a time in an intrauterine environment and receive nourishment and symbionts in mother's milk. We show that the host peptidoglycan recognition protein (PGRP-LB) is expressed only in adults and is a major component of the milk that nourishes the developing progeny. The amidase activity associated with PGRP-LB may scavenge the symbiotic peptidoglycan and prevent the induction of tsetse's immune deficiency pathway that otherwise can damage the symbionts. Reduction of PGRP-LB experimentally diminishes female fecundity and damages *Wigglesworthia* in the milk through induction of antimicrobial peptides, including attacin. Larvae that receive less maternal PGRP-LB give rise to adults with fewer *Wigglesworthia* and hyperimmune responses. Such adults also suffer dysregulated immunity, as indicated by the presence of higher trypanosome densities in parasitized adults. We show that recPGRP-LB has antimicrobial and antitrypanosomal activities that may regulate symbiosis and impact immunity. Thus, PGRP-LB plays a pivotal role in tsetse's fitness by protecting symbiosis against host-inflicted damage during development and by controlling parasite infections in adults that can otherwise reduce host fecundity.

16078. **Weiss, B. L., Maltz, M. & Aksoy, S., 2012.** Obligate symbionts activate immune system development in the tsetse fly. *Journal of Immunology*, **188** (7): 3395-3403.

Division of Epidemiology of Microbial Diseases, Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, CT 06520, USA. [brian.weiss@yale.edu].

Many insects rely on the presence of symbiotic bacteria for proper immune system function. However, the molecular mechanisms that underlie this phenomenon are poorly understood. Adult tsetse flies (*Glossina* spp.) house three symbiotic bacteria that are vertically transmitted from mother to offspring during this insect's unique viviparous mode of reproduction. Larval tsetse that undergo intrauterine development in the absence of their obligate mutualist *Wigglesworthia*, exhibit a compromised immune system during adulthood.

In this study, we characterize the immune phenotype of tsetse that develops in the absence of all of their endogenous symbiotic microbes. Aposymbiotic tsetse (*Glossina morsitans morsitans* [Gmm(Apo)]) present a severely compromised immune system that is characterized by the absence of phagocytic haemocytes and atypical expression of immunity-related genes. Correspondingly, these flies quickly succumb to infection with normally non-pathogenic *Escherichia coli*. The susceptible phenotype exhibited by Gmm(Apo) adults can be reversed when they receive haemocytes transplanted from wild-type donor flies prior to infection. Furthermore, the process of immune system development can be restored in intrauterine Gmm(Apo) larvae when their mothers are fed a diet supplemented with *Wigglesworthia* cell extracts. Our finding that molecular components of *Wigglesworthia* exhibit immunostimulatory activity within tsetse is representative of a novel evolutionary adaptation that steadfastly links an obligate symbiont with its host.

3. TSETSE CONTROL (INCLUDING ENVIRONMENTAL SIDE EFFECTS)

[See also **35**:16071, 16072, 16073, 16075, 16076]

16079. **Gechere, G., Terefe, G. & Belihu, K., 2012.** Impact of tsetse and trypanosomiasis control on cattle herd composition and calf growth and mortality at Arbaminch District (Southern Rift Valley, Ethiopia). *Tropical Animal Health & Production*. **E publication ahead of print, April 1.**

Kality Tsetse Rearing and Irradiation Center, Ministry of Science and Technology, P.O. Box. 19917, Addis Ababa, Ethiopia; School of Veterinary Medicine, Addis Ababa University, P.O. Box. 34, Debre Zeit, Ethiopia; and FAO-ETH, Awash Field Office, Awash, Ethiopia. [getachew_terefe@yahoo.com].

The effect of tsetse/trypanosomiasis control on cattle herd composition and growth and mortality of calves in tsetse controlled (by southern tsetse eradication project [STEP]) and uncontrolled blocks in southern Ethiopia was assessed. A structured questionnaire was used to interview 182 households to estimate cattle herd composition and calf mortality. Calves were bled to examine the presence of trypanosomes by the buffy coat technique. Forty NGU traps were deployed and fly catches determined. A case-control study was performed on 40 calves for 6 months to estimate calf growth parameters. The mean cattle herd size was lower in the tsetse-controlled block than in the uncontrolled block, whereas the relative number of calves in a herd tended to be higher in the tsetse-controlled block ($P = 0.06$). While there was no report of cattle mortality in the tsetse-controlled block, 16.48 percent of the respondents lost calves in tsetse-uncontrolled block over a one year period. The prevalence of trypanosome positive calves was 2.95 percent for the uncontrolled block but there were no positive cases in the tsetse-controlled block. The apparent densities of flies/trap/day in the tsetse-uncontrolled block were 30-fold higher than in the tsetse-controlled block ($P < 0.01$). The case-control study revealed that the mean body weight gain of calves in the tsetse-controlled block (40.23 +/- 0.7 kg) was significantly higher than that of the uncontrolled block (34.74 +/- 0.68 kg). The above findings strongly suggest that the intervention by the STEP project has significantly reduced the tsetse population and trypanosomiasis, thereby contributing to improved calf growth and survival.

16080. **Hargrove, J. W., Ouifki, R., Kajunguri, D., Vale, G. A. & Torr, S. J., 2012.** Modelling the control of trypanosomiasis using trypanocides or insecticide-treated livestock. *PLoS Neglected Tropical Diseases*, **6** (5): e1615.

DST/NRF Centre of Excellence in Epidemiological Modelling and Analysis (SACEMA), University of Stellenbosch, Stellenbosch, South Africa; and Natural Resources Institute, University of Greenwich, Chatham Maritime, UK [jhargrove@sun.ac.za].

In Uganda, Rhodesian sleeping sickness, caused by *Trypanosoma brucei rhodesiense*, and animal trypanosomiasis caused by *T. vivax* and *T. congolense*, are being controlled by treating cattle with trypanocides and/or insecticides. We used a mathematical model to identify treatment coverages required to break transmission when host populations consisted of various proportions of wild and domestic mammals, and reptiles. An R_0 model for trypanosomiasis was generalized to allow tsetse to feed off multiple host species. Assuming populations of cattle and humans only, pre-intervention R_0 values for *T. vivax, T. congolense*, and *T. brucei* were 388, 64 and 3, respectively. Treating cattle with trypanocides reduced R_0 for *T. brucei* to <1 if >65 percent of cattle were treated, vs 100 percent coverage necessary for *T. vivax* and *T. congolense*. The presence of wild mammalian hosts increased the coverage required and made control of *T. vivax* and *T. congolense* impossible. When tsetse fed only on cattle or humans, the R_0 for *T. brucei* was <1 if 20 percent of cattle were treated with insecticide, compared with 55 percent for *T. congolense*. If wild mammalian hosts were also present, control of the two species was impossible if proportions of non-human bloodmeals from cattle were <40 percent or <70 percent, respectively. R_0 was <1 for *T. vivax* only when insecticide treatment led to reductions in the tsetse population. Under such circumstances the R_0 was <1 for *T. brucei* and *T. congolense* if cattle make up 30 percent and 55 percent, respectively of the non-human tsetse bloodmeals, as long as all cattle are treated with insecticide. The results suggest that in settled areas of Uganda with few wild hosts, Rhodesian sleeping sickness is likely to be much more effectively controlled by treating cattle with insecticide than with trypanocides.

16081. **Lindh, J. M., Goswami, P., Blackburn, R. S., Arnold, S. E., Vale, G. A., Lehane, M. J. & Torr, S. J., 2012.** Optimizing the colour and fabric of targets for the control of the tsetse fly *Glossina fuscipes fuscipes*. *PLoS Neglected Tropical Diseases*, **6** (5): e1661.

Vector Group, Liverpool School of Tropical Medicine, Liverpool, UK; African Insect Science for Food and Health, Thomas Odhiambo Campus, Mbita Point, Kenya; Sustainable Materials Research Group, Centre for Technical Textiles, University of Leeds, Leeds, UK; Natural Resources Institute, University of Greenwich, Chatham Maritime, UK; and Southern African Centre for Epidemiological Modelling and Analysis, University of Stellenbosch, Stellenbosch, South Africa. [m.j.lehane@liv.ac.uk].

Most cases of human African trypanosomiasis (HAT) start with a bite from one of the subspecies of *Glossina fuscipes*. Tsetse use a range of olfactory and visual stimuli to locate their hosts and this response can be exploited to lure tsetse to insecticide-treated targets thereby reducing transmission. To provide a rational basis for cost-effective designs of target, we undertook studies to identify the optimal target colour. On the Chamaunga islands of Lake Victoria, Kenya, studies were made of the numbers of *G. fuscipes fuscipes* attracted to targets consisting of a panel (25 cm^2) of various coloured fabrics flanked by a panel (also 25 cm^2) of fine black netting. Both panels were covered with an electrocuting grid to catch tsetse as they contacted the target. The reflectances of the 37 different-coloured cloth panels utilised in the study were measured spectrophotometrically. Catch was positively correlated with percentage

reflectance at the blue (460 nm) wavelength and negatively correlated with reflectance at UV (360 nm) and green (520 nm) wavelengths. The best target was subjectively blue, with percentage reflectances of 3 percent, 29 percent, and 20 percent at 360 nm, 460 nm and 520 nm respectively. The worst target was also, subjectively, blue, but with high reflectances at UV (35 percent reflectance at 360 nm) wavelengths as well as blue (36 percent reflectance at 460 nm); the best low UV-reflecting blue caught three times more tsetse than the high UV-reflecting blue. It is concluded that insecticide-treated targets to control *G. f. fuscipes* should be blue with low reflectance in both the UV and green bands of the spectrum. Targets that are subjectively blue will perform poorly if they also reflect UV strongly. The selection of fabrics for targets should be guided by spectral analysis of the cloth across both the spectrum visible to humans and the UV region.

16082. **McCord, P. F., Messina, J. P., Campbell, D. J. & Grady, S. C., 2012.** Tsetse fly control in Kenya's spatially and temporally dynamic control reservoirs: a cost analysis. *Applied Geography*, **34**: 189-204.

Department of Geography, Center for Global Change and Earth Observations, Michigan State University, 218 Manly Miles Building, 1405 S. Harrison Road, East Lansing, Michigan 48823, USA. [mccordpa@msu.edu].

Human African trypanosomiasis (HAT) and animal African trypanosomiasis (AAT) are significant health concerns throughout much of sub-Saharan Africa. Funding for tsetse fly control operations has decreased since the 1970s, which has in turn limited the success of campaigns to control the disease vector. To maximize the effectiveness of the limited financial resources available for tsetse control, this study develops and analyses spatially and temporally dynamic tsetse distribution maps of *Glossina* subgenus *morsitans* populations in Kenya from January 2002 to December 2010, produced using the Tsetse Ecological Distribution Model. These species distribution maps reveal seasonal variations in fly distributions. Such variations allow for the identification of "control reservoirs" where fly distributions are spatially constrained by fluctuations in suitable habitat and tsetse population characteristics. Following identification of the control reservoirs, a tsetse management operation is simulated in the control reservoirs using capital and labour control inputs from previous studies. Finally, a cost analysis, following specific economic guidelines from existing tsetse control analyses, is conducted to calculate the total cost of a nationwide control campaign of the reservoirs compared to the cost of a nationwide campaign conducted at the maximum spatial extent of the fly distributions from January 2002 to December 2010. The total cost of tsetse management within the reservoirs comes to US$14 212 647, while the nationwide campaign at the maximum spatial extent amounts to US$33 721 516. This saving of US$19 508 869 illustrates the importance of identifying seasonally dynamic control reservoirs when conducting a tsetse management campaign, and in the process offers an economical means of fly control and disease management for future programme planning.

16083. **Rayaisse, J. B., Krober, T., McMullin, A., Solano, P., Mihok, S. & Guerin, P. M., 2012.** Standardizing visual control devices for tsetse flies: West African species *Glossina tachinoides, G. palpalis gambiensis* and *G. morsitans submorsitans*. *PLoS Neglected Tropical Diseases*, **6** (2): e1491.

Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), Bobo-Dioulasso, Burkina Faso.

Here we describe field trials designed to standardize tools for the control of *Glossina*

tachinoides, G. palpalis gambiensis and *G. morsitans submorsitans* in West Africa based on existing trap/target/bait technology. Blue and black biconical and monoconical traps and 1 m^2 targets were made using either phthalogen blue cotton, phthalogen blue cotton/polyester or turquoise blue polyester/viscose (all with a peak reflectance between 450-480 nm) and a black polyester. Because targets were covered in adhesive film, they proved to be significantly better trapping devices than either of the two trap types for all three species (up to 14 times more for *G. tachinoides*, 10 times more for *G. palpalis gambiensis*, and 6.5 times for *G. morsitans submorsitans*). The relative performance of the devices in the three blue cloths tested was the same when unbaited or baited with a mixture of phenols, 1-octen-3-ol and acetone. Since insecticide-impregnated devices act via contact with flies, we enumerated which device (traps or targets) served as the best object for flies to land on by also covering the cloth parts of the traps with adhesive film. Despite the fact that the biconical trap proved to be the best landing device for the three species, the difference over the target (20-30 percent) was not significant. This experiment also allowed an estimation of trap efficiency, i.e. the proportion of flies landing on a trap that are caught in its cage. A low overall efficiency of the biconical or monoconical traps of between 11-24 percent was recorded for all three species. These results show that targets can be used as practical devices for population suppression of the three species studied. Biconical traps can be used for population monitoring, but a correction factor of 5-10 fold needs to be applied to captures to compensate for the poor trapping efficiency of this device for the three species.

16084. **Sow, A., Sidibe, I., Bengaly, Z., Bance, A. Z., Sawadogo, G. J., Solano, P., Vreysen, M. J., Lancelot, R. & Bouyer, J., 2012.** Irradiated male tsetse from a 40-year-old colony are still competitive in a riparian forest in Burkina Faso. *PLoS One*, **7** (5): e37124.

Centre International de Recherche-Développement sur l'Elevage en Zone Subhumide, Bobo-Dioulasso, Burkina Faso. [bouyer@cirad.fr].

Tsetse flies are the cyclical vectors of African trypanosomosis which constitutes a major constraint to development in Africa. Their control is an important component of the integrated management of these diseases, and among the techniques available, the sterile insect technique (SIT) is the most efficient at low densities. The government of Burkina Faso has embarked on a tsetse eradication programme within the framework of the PATTEC, where SIT is an important component. The project plans to use flies from a *Glossina palpalis gambiensis* colony that has been maintained for about 40 years at the Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES). It was thus necessary to test the competitiveness of the sterile males originating from this colony. During the period January-February 2010, 16 000 sterile male *G. p. gambiensis* were released along a tributary of the Mouhoun river. The study revealed that with a mean sterile to wild male ratio of 1.16 (S.D. 0.38), the abortion rate of the wild female flies was significantly higher than before ($P = 0.026$) and after ($P = 0.019$) the release period. The estimated competitiveness of the sterile males (Fried index) was 0.07 (S.D. 0.02), indicating that a sterile to wild male ratio of 14.4 would be necessary to obtain nearly complete induced sterility in the female population. The aggregation patterns of sterile and wild male flies were similar. The survival rate of the released sterile male flies was similar to that observed in 1983-1985 for the same colony. We conclude that gamma sterilised male *G. p. gambiensis* derived from the CIRDES colony have a competitiveness that is comparable with their competitiveness obtained 35 years ago and can still be used for an area-wide integrated pest management campaign with a sterile insect component in Burkina Faso.

16085. **Taye, M., Belihu, K., Bekana, M. & Sheferaw, D., 2012.** Assessment of impacts of tsetse and trypanosomosis control measures on cattle herd composition and performance in southern region, Ethiopia. *Tropical Animal Health & Production*. **E publication ahead of print, April 14.**

College of Agriculture, Arba-Minch University, P.O. Box 21, Arba-Minch, Ethiopia.

This study was conducted to assess the impact of tsetse and trypanosomosis control measures on cattle herd size and composition, herd dynamics, and milk yield in Wolaita and Gamogofa Zones, southern Ethiopia. The study showed that the average cattle herd size in tsetse challenged areas was significantly higher than those in tsetse-controlled areas. The number of non-pregnant dry cows, bulls and oxen were significantly higher in tsetse challenged areas than the other two study areas. The rate of cattle addition to and disposal from the herd were significantly higher in tsetse challenged areas. Cows in the Southern Tsetse Eradication Project (STEP) and community tsetse controlled areas were able to give 26-27 percent, 25-29 percent and 17-21 percent more daily milk yield at the beginning, middle and end of lactation respectively, than those in tsetse-challenged areas. In addition, cows in STEP and community tsetse controlled areas had lactation lengths that were longer by 1.20 to 1.35 months; age at first calving was shorter by 5.30 to 5.10 months; and calving interval was shorter by 4.20 to 3.20 months than cows in tsetse-challenged area, respectively. Hence, tsetse and trypanosomosis control both by the community and project would play key roles in the improvement of cattle productivity.

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

[See also **35**: 16053, 16068, 16069, 16070, 16071]

16086. **Alam, U., Hyseni, C., Symula, R. E., Brelsfoard, C., Wu, Y., Kruglov, O., Wang, J., Echodu, R., Alioni, V., Okedi, L. M., Caccone, A. & Aksoy, S., 2012.** Implications of microfauna-host interactions for trypanosome transmission dynamics in *Glossina fuscipes fuscipes* in Uganda. *Applied & Environmental Microbiology*, **78** (13): 4627-4637.

Yale University School of Public Health, New Haven, Connecticut, USA; Department of Ecology and Evolutionary Biology, Yale University, New Haven, Connecticut, USA; and National Livestock Resources Research Institute, Tororo, Uganda. [serap.aksoy@yale.edu].

Tsetse flies (Diptera: Glossinidae) are vectors for African trypanosomes (Euglenozoa: kinetoplastida), protozoan parasites that cause African trypanosomiasis in humans (HAT) and nagana in livestock. In addition to trypanosomes, two symbiotic bacteria (*Wigglesworthia glossinidia* and *Sodalis glossinidius*) and two parasitic microbes, *Wolbachia* and a salivary gland hypertrophy virus (SGHV), have been described in tsetse. Here we determined the prevalence of and coinfection dynamics between *Wolbachia*, trypanosomes, and SGHV in *Glossina fuscipes fuscipes* in Uganda over a large geographical scale spanning the range of host genetic and spatial diversity. Using a multivariate analysis approach, we uncovered complex coinfection dynamics between the pathogens and statistically significant associations between host genetic groups and pathogen prevalence. It is important to note that these coinfection dynamics and associations with the host were not apparent by univariate analysis.

These associations between host genotype and pathogen are particularly evident for *Wolbachia* and SGHV where host groups are inversely correlated for *Wolbachia* and SGHV prevalence. On the other hand, trypanosome infection prevalence is more complex and covaries with the presence of the other two pathogens, highlighting the importance of examining multiple pathogens simultaneously before making generalizations about infection and spatial patterns. It is imperative to note that these novel findings would have been missed if we had employed the standard univariate analysis used in previous studies. Our results are discussed in the context of disease epidemiology and vector control.

16087. **Auty, H. K., Picozzi, K., Malele, I., Torr, S. J., Cleaveland, S. & Welburn, S., 2012.** Using molecular data for epidemiological inference: assessing the prevalence of *Trypanosoma brucei rhodesiense* in tsetse in Serengeti, Tanzania. *PLoS Neglected Tropical Diseases*, **6** (1): e1501.

Division of Pathway Medicine and Centre for Infectious Diseases, School of Biomedical Sciences, College of Medicine and Veterinary Medicine, The University of Edinburgh, Edinburgh, UK; Institute for Biodiversity, Animal Health and Comparative Medicine, College of Medicine, Veterinary Medicine and Life Sciences, University of Glasgow, Glasgow, UK; Tsetse and Trypanosomiasis Research Institute, Tanga, Tanzania; and Natural Resources Institute, University of Greenwich, Chatham Maritime, UK [sue.welburn@ed.ac.uk].

Measuring the prevalence of transmissible *Trypanosoma brucei rhodesiense* in tsetse populations is essential for understanding transmission dynamics, assessing human disease risk and monitoring spatio-temporal trends and the impact of control interventions. Although an important epidemiological variable, identifying flies which carry transmissible infections is difficult, with challenges including low prevalence, presence of other trypanosome species in the same fly, and concurrent detection of immature non-transmissible infections. Diagnostic tests to measure the prevalence of *T. b. rhodesiense* in tsetse are applied and interpreted inconsistently, and discrepancies between studies suggest this value is not consistently estimated even to within an order of magnitude. Three approaches were used to estimate the prevalence of transmissible *Trypanosoma brucei s.l.* and *T. b. rhodesiense* in *Glossina swynnertoni* and *G. pallidipes* in the Serengeti National Park, Tanzania: (i) dissection/microscopy; (ii) PCR on infected tsetse midguts; and (iii) inference from a mathematical model. Using dissection/microscopy the prevalence of transmissible *T. brucei s.l.* was 0 percent (95 percent CI 0-0.085) for *G. swynnertoni* and 0 percent (0-0.18) for *G. pallidipes*; using PCR the prevalence of transmissible *T. b. rhodesiense* was 0.010 percent (0-0.054) and 0.0089 percent (0-0.059) respectively, and by model inference 0.0064 percent and 0.00085 percent respectively. The zero prevalence result by dissection/microscopy (likely really greater than zero given the results of other approaches) is not unusual by this technique, often ascribed to poor sensitivity. The application of additional techniques confirmed the very low prevalence of *T. brucei* suggesting the zero prevalence result was attributable to insufficient sample size (despite examination of 6 000 tsetse). Given the prohibitively high sample sizes required to obtain meaningful results by dissection/microscopy, PCR-based approaches offer the current best option for assessing trypanosome prevalence in tsetse but inconsistencies in relating PCR results to transmissibility highlight the need for a consensus approach to generate meaningful and comparable data.

16088. **De Vooght, L., Caljon, G., Stijlemans, B., De Baetselier, P., Coosemans, M. & Van den Abbeele, J., 2012.** Expression and extracellular release of a functional anti-trypanosome Nanobody® in *Sodalis glossinidius*, a bacterial symbiont of the tsetse fly.

Microbial Cell Factories, **11**: 23.

Department of Biomedical Sciences, Unit of Veterinary Protozoology, Institute of Tropical Medicine Antwerp, Antwerp, Belgium; Unit of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium; and Laboratory of Myeloid Cell Immunology, VIB, Brussels, Belgium.

Sodalis glossinidius, a gram-negative bacterial endosymbiont of the tsetse fly, has been proposed as a potential *in vivo* drug delivery vehicle to control trypanosome parasite development in the fly, an approach known as paratransgenesis. Despite this interest in *S. glossinidius* as a paratransgenic platform organism in tsetse flies, few potential effector molecules have been identified so far and to date none of these molecules has been successfully expressed in this bacterium. In this study, *S. glossinidius* was transformed to express a single domain antibody, (Nanobody®) Nb_An33, that efficiently targets conserved cryptic epitopes of the variant surface glycoprotein (VSG) of the parasite *Trypanosoma brucei*. Next, we analysed the capability of two predicted secretion signals to direct the extracellular delivery of significant levels of active Nb_An33. We show that the pelB leader peptide was successful in directing the export of fully functional Nb_An33 to the periplasm of *S. glossinidius* resulting in significant levels of extracellular release. Finally, *S. glossinidius* expressing pelBNb_An33 exhibited no significant reduction in terms of fitness, determined by *in vitro* growth kinetics, compared with the wild-type strain. These data are the first demonstration of the expression and extracellular release of functional trypanosome-interfering Nanobodies® in *S. glossinidius*. Furthermore, *Sodalis* strains that efficiently released the effector protein were not affected in their growth, suggesting that they may be competitive with endogenous microbiota in the midgut environment of the tsetse fly. Collectively, these data reinforce the notion for the potential of *S. glossinidius* to be developed into a paratransgenic platform organism.

16089. **Munang'andu, H. M., Siamudaala, V., Munyeme, M. & Nalubamba, K. S., 2012.** A review of ecological factors associated with the epidemiology of wildlife trypanosomiasis in the Luangwa and Zambezi valley ecosystems of Zambia. *Interdisciplinary Perspectives on Infectious Diseases*, **2012**: 372523.

Section of Aquatic Medicine and Nutrition, Department of Basic Sciences and Aquatic Medicine, Norwegian School of Veterinary Sciences, Ullevalsveien 72, P.O. Box 8146 Dep, 0033 Oslo, Norway; Kavango Zambezi Transfrontier Conservation Area Secretariat, Kasane 821, Gaborone, Botswana; Department of Disease Control, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia;; and Department of Clinical Studies, School of Veterinary Medicine, University of Zambia, P.O. Box 32379, Lusaka 10101, Zambia.

Trypanosomiasis has been endemic in wildlife in Zambia for more than a century. The disease has been associated with neurological disorders in humans. Current conservation strategies by the Zambian government of turning all game reserves into state-protected National Parks (NPs) and game management areas (GMAs) have led to the expansion of the wildlife and tsetse population in the Luangwa and Zambezi valley ecosystems. This ecological niche lies in the common tsetse fly belt that harbours the highest tsetse population density in Southern Africa. Ecological factors such as climate, vegetation and rainfall found in this niche allow for a favourable interplay between wild reservoir hosts and vector tsetse flies. These ecological factors that influence the survival of a wide range of wildlife species provide adequate habitat for tsetse flies thereby supporting the coexistence of disease

reservoir hosts and vector tsetse flies leading to prolonged persistence of trypanosomiasis in the area. On the other hand, increase in anthropogenic activities poses a significant threat of reducing the tsetse and wildlife habitat in the area. Herein, we demonstrate that while conservation of wildlife and biodiversity is an important preservation strategy of natural resources, it could serve as a long-term reservoir of wildlife trypanosomiasis.

16090. **Peacock, L., Cook, S., Ferris, V., Bailey, M. & Gibson, W., 2012.** The life cycle of *Trypanosoma (Nannomonas) congolense* in the tsetse fly. *Parasites & Vectors*, **5**: 109.

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

The tsetse-transmitted African trypanosomes cause diseases of importance to the health of both humans and livestock. The life cycles of these trypanosomes in the fly were described in the last century, but comparatively few details are available for *Trypanosoma (Nannomonas) congolense*, despite the fact that it is probably the most prevalent and widespread pathogenic species for livestock in tropical Africa. When the fly takes up bloodstream form trypanosomes, the initial establishment of midgut infection and invasion of the proventriculus is much the same in *T. congolense* and *T. brucei*. However, the developmental pathways subsequently diverge, with production of infective metacyclics in the proboscis for *T. congolense* and in the salivary glands for *T. brucei*. Whereas events during migration from the proventriculus are understood for *T. brucei*, knowledge of the corresponding developmental pathway in *T. congolense* is rudimentary. The recent publication of the genome sequence makes it timely to re-investigate the life cycle of *T. congolense*. Experimental tsetse flies were fed an initial bloodmeal containing *T. congolense* strain 1/148 and dissected 2 to 78 days later. Trypanosomes recovered from the midgut, proventriculus, proboscis and cibarium were fixed and stained for digital image analysis. Trypanosomes contained in spit samples from individually caged flies were analysed similarly. Mensural data from individual trypanosomes were subjected to principal components analysis. Flies were more susceptible to infection with *T. congolense* than *T. brucei*; a high proportion of flies infected with *T. congolense* established a midgut and subsequent proboscis infection, whereas many *T. brucei* infections were lost in the migration from foregut to salivary glands. In *T. congolense*, trypomastigotes ceased division in the proventriculus and became uniform in size. The trypanosomes retained trypomastigote morphology during migration via the foregut to the mouthparts and we confirmed that the trypomastigote-epimastigote transition occurred in the proboscis. We found no equivalent to the asymmetric division stage in *T. brucei* that mediates transition of proventricular trypomastigotes to epimastigotes. In *T. congolense* extremely long epimastigotes with remarkably elongated posterior ends were observed in both the proboscis and cibarium; no difference was found in the developmental stages in these two organs. Dividing trypomastigotes and epimastigotes were recovered from the proboscis, some of which were in transition from trypomastigote to epimastigote and vice versa. It remains uncertain whether these morphological transitions are mediated by cell division, since we also found non-dividing cells with a variously positioned, juxta-nuclear kinetoplast. In conclusion, we have presented a detailed description of the life cycle of *T. congolense* in its tsetse fly vector. During development in the fly *T. congolense* shares a common migratory pathway with its close relative *T. brucei*, culminating in the production of small metacyclic trypanosomes that can be inoculated with the saliva. Despite this outward similarity in life cycle, the transitional developmental stages in the foregut and mouthparts are remarkably different in the two trypanosome species.

16091. **Peacock, L., Ferris, V., Bailey, M. & Gibson, W., 2012.** The influence of sex and fly species on the development of trypanosomes in tsetse flies. *PLoS Neglected Tropical Diseases*, **6** (2): e1515.

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

Unlike other dipteran disease vectors, tsetse flies of both sexes feed on blood and transmit pathogenic African trypanosomes. During transmission, *Trypanosoma brucei* undergoes a complex cycle of proliferation and development inside the tsetse vector, culminating in production of infective forms in the saliva. The insect manifests robust immune defences throughout the alimentary tract, which eliminate many trypanosome infections. Previous work has shown that fly sex influences susceptibility to trypanosome infection as males show higher rates of salivary gland (SG) infection with *T. brucei* than females. To investigate sex-linked differences in the progression of infection, we compared midgut (MG), proventriculus, foregut and SG infections in male and female *Glossina morsitans morsitans*. Initially, infections developed in the same way in both sexes: no difference was observed in numbers of MG or proventriculus infections, or in the number and type of developmental forms produced. Female flies tended to produce foregut migratory forms later than males, but this had no detectable impact on the number of SG infections. The sex difference was not apparent until the final stage of SG invasion and colonisation, showing that the SG environment differs between male and female flies. Comparison of *G. m. morsitans* with *G. pallidipes* showed a similar, though less pronounced, sex difference in susceptibility, but additionally revealed very different levels of trypanosome resistance in the MG and SG. While G. *pallidipes* was more refractory to MG infection, a very high proportion of MG infections led to SG infection in both sexes. It appears that the two fly species use different strategies to block trypanosome infection: *G. pallidipes* heavily defends against initial establishment in the MG, while *G. m. morsitans* has additional measures to prevent trypanosomes colonising the SG, particularly in female flies. We conclude that the tsetse-trypanosome interface works differently in *G. m. morsitans* and *G. pallidipes.*

16092. **Rodriguez, N. F., Tejedor-Junco, M. T., Gonzalez-Martin, M., Santana del Pino, A. & Gutierrez, C., 2012.** Cross-sectional study on prevalence of *Trypanosoma evansi* infection in domestic ruminants in an endemic area of the Canary Islands (Spain). *Preventive Veterinary Medicine*, **105** (1-2): 144-148.

Department of Animal Medicine and Surgery, Veterinary Faculty, University of Las Palmas de Gran Canaria, 35413 Arucas, Las Palmas, Canary Islands, Spain. [mtejedor@dcc.ulpgc.es].

Trypanosoma evansi is the most widely spread of the pathogenic African trypanosomes of animals. The disease (surra) was first diagnosed in the Canary Islands in a dromedary camel in 1997; thus, a control plan was implemented achieving the eventual eradication of *T. evansi* from most of the infected areas in the Archipelago. However, a little area remains still infected despite the use of the same control measures. To evaluate possible reservoirs in the area a representative sample of domestic ruminants was examined by serological, parasitological and molecular tests. Of a total of 1 228 ruminants assessed, 61 (5 percent) were serologically positive (7 cattle, 21 goats, 33 sheep), but *T. evansi* could be demonstrated in none of them. According to FreeCalc assessment, cattle and goat populations would be free from disease; however, the results from sheep are not adequate to conclude that the population would be free from disease. As a conclusion, surveillance must be conducted on

ruminant farms in the surroundings of the infected area in order to evaluate the possible extension of the disease and their potential role as reservoirs of *T. evansi*.

16093. **Simo, G., Njitchouang, G. R., Njiokou, F., Cuny, G. & Asonganyi, T., 2012.** Genetic characterization of *Trypanosoma brucei* circulating in domestic animals of the Fontem sleeping sickness focus of Cameroon. *Microbes & Infection*, **14** (7-8): 651-658.

Department of Biochemistry, Faculty of Science, P.O. Box 67, University of Dschang, Dschang, Cameroon; General Biology Laboratory, Department of Biology and Animal Physiology, Faculty of Science, P.O. Box 812, University of Yaoundé 1, Cameroon; Laboratoire de Recherche et de Coordination sur les Trypanosomoses IRD, UMR 177, CIRAD, TA 207/G Campus International de Baillarguet, 34398 Montpellier Cedex 5, France; and Faculty of Medicine and Biomedical Sciences, University of Yaoundé 1, Cameroon. [gsimoca@yahoo.fr].

To improve our knowledge on the transmission dynamics of trypanosomes, *Trypanosoma brucei* was identified in domestic animals of the Fontem sleeping sickness focus of Cameroon and its genetic characterization was performed using seven polymorphic microsatellite markers. About 397 domestic animals including 225 pigs, 87 goats, 65 sheep and 20 dogs were sampled. The card agglutination test for trypanosomiasis was positive for 254 (63.98 percent) animals while the parasitological examinations (thin blood film and capillary tube centrifugation) revealed 86 (21.66 percent) trypanosome infections. The PCR based method revealed 140 (35.26 percent) infections of trypanosomes of the subgenus Trypanozoon. The genetic characterization of these 140 positive samples revealed 89 different alleles: 82 in pigs, 72 in goat, 60 in sheep and 48 in dog. Whatever the microsatellite marker used, most of positive samples were amplified. However, the sensitivity (percentage of samples amplified for each marker) of these markers varied significantly between them ($chi^2 = 120.32$; $P < 0.0001$). This study showed a high level (80.00 percent) of mixed genotypes as well as a wide range of *T. brucei* genotypes circulating in domestic animals of the Fontem sleeping sickness focus of Cameroon. This indicates that several *T. brucei* genotypes can naturally be transmitted simultaneously to tsetse flies during a single blood meal.

16094. **Simon, F., Mura, M., Pages, F., Morand, G., Truc, P., Louis, F. & Gautret, P., 2012.** Urban transmission of human African trypanosomiasis, Gabon. *Emerging Infectious Diseases*, **18** (1): 165-167.

Hôpital d'Instruction des Armées Laveran, Marseille, France; Institut de Médecine Tropicale du Service de Santé des Armées, Marseille, France; Institut de Recherche pour le Développement, Montpellier, France; and Organisation de Coordination pour la Lutte contre les Endémies en Afrique Centrale, Yaoundé, Cameroon.

We describe a confirmed case of human African trypanosomiasis (HAT) in an expatriate returning to France from Gabon after a probable tsetse fly bite in the urban setting of Libreville. This case indicates a possible urban transmission of HAT in Gabon and stresses the need for entomologic studies in Libreville. HAT is endemic to sub-Saharan Africa. *Trypanosoma brucei rhodesiense* (eastern Africa) and *T. b. gambiense* (western Africa) parasites are transmitted to humans by tsetse flies of the *Glossina morsitans* group (*T .b. rhodesiense*) and of the *G. palpalis* group (*T. b. gambiense*), which are found only in Africa. *T. b. gambiense* represents >90 percent of all reported cases of HAT worldwide. HAT has always been a travel-associated disease. It is a rare cause of fever, cutaneous lesions, and

neurologic signs in travellers returning from disease-endemic areas and involves *T. b. rhodesiense* in 70 percent of the cases, resulting mostly from an exposure during safari in game parks. A 58-year-old previously healthy Portuguese man who worked in Gabon for 13 years for a French company was admitted to the tropical and infectious diseases ward because of a two-month history of intermittent fever, fatigue, and a 10-kg weight loss. The patient recalled a painful unidentified insect bite on his right thigh two months before in his garden in Libreville (Lalala quarter). An 8-cm, indurated, erythematous, and painful plaque (chancre) progressively developed during the following weeks after the assumed insect bite. When admitted to the hospital, the patient had a temperature of 39 °C, anorexia, insomnia, pruritus of the left arm, and paresthesia of the hands and feet. Two additional large annular erythematous macules, centrally pale (trypanids), were found on his back. A subclavicular 0.5 cm lymph node was observed. There was no hepatosplenomegaly. His laboratory results showed moderate anaemia (haemoglobin 11.8 g/dL) and thrombopaenia (134 000 platelets/mm^3) and elevated levels of C-reactive protein (30.6 mg/L) and gammaglobulins (23.9 g/L). A thick-blood smear showed no malaria parasites but a few trypomastigotes of *Trypanosoma* spp. PCR of blood identified *T. b. gambiense.* A cerebrospinal fluid sample showed moderate elevation of total proteins (0.43 g/L) and albumin (291 mg/L), 11 leukocytes, and no IgM elevation. Direct examination and PCR showed no trypanosome in the cerebrospinal fluid. Specific antibodies were found in the blood by indirect immunofluorescence (titre 200). Biopsies of two skin lesions (thigh, back) showed a lymphoplasmocytic vasculitis consistent with cutaneous locations of HAT; no parasite was observed *in situ*. The patient was treated successfully with a 7-day course of pentamidine. The case was reported to World Health Organization Control of Neglected Tropical Diseases Department. A total of 328 HAT cases were reported to the World Health Organization in Gabon during 2000–2009; most infections were acquired in the mangrove swamp Atlantic coast focus in Noya (Estuaire Province) and some in the focus of Bendje (Ogooué-Maritime Province). Four of six cases of *T. b. gambiense* imported to Europe during 2005–2009 were in expatriates with a travel history to Gabon. In the four case-patients infected in Gabon, an exposure in rural forest areas was assessed (D. Malvy, pers. comm.). In the fifth case reported here, the tsetse bite likely occurred in the urban setting of Libreville. The patient did not report occupational exposure to tsetse bites outside Libreville during the previous year. He occasionally went in Pointe Denis during weekends but did not remember having been bitten by a tsetse fly. Although the patient did not identify the insect in his garden, the chronology of his clinical history and the presence of a typical chancre at the place of the insect bite that occurred before symptoms provide strong arguments in favour of this hypothesis. The bite occurred during the morning hours, in the patient's home garden in the Lalala area of Libreville (0.357568N, 9.475365E) near the Ogombié River. This area is located 125 km and 75 km from the Bendje and Noya HAT foci, respectively. Two studies provided evidence for urban transmission of HAT in Kinshasa (Democratic Republic of Congo) and in Bonon (Côte d'Ivoire). Concurrently, some tsetse species such as *G. palpalis*, adapt to high human densities and are found in the largest urban centres of western Africa. Entomologic studies in Libreville should prompt further investigation into a possible urban transmission of HAT in Gabon, as we suspect in the case reported.

5. HUMAN TRYPANOSOMOSIS

(a) SURVEILLANCE

[See also **35**: 16039, 16040, 16057]

16095. **Corral-Corral, I. & Quereda Rodriguez-Navarro, C., 2012.** Gustavo Pittaluga and the expedition to study sleeping sickness in the Spanish territories of the Gulf of Guinea (1909). *Revue Neurologique*, **54** (1): 49-58.

Servicio de Neurologia, Hospital Universitario Ramon y Cajal, 28034 Madrid, Espana. [icorral.hrc@salud.madrid.org].

Sleeping sickness, or human African trypanosomiasis, caused important mortality at the beginning of the twentieth century. For this reason the European colonial countries organized several scientific expeditions which contributed decisively to the knowledge of the disease. The aim of this paper is to study the first investigation performed in Spain on African trypanosomiasis and in the field of tropical medicine, which was accomplished by a scientific expedition to the Spanish territories in the Gulf of Guinea organized by Cajal in 1909. The parasitologist Gustavo Pittaluga, who became one of the most outstanding figures in Spanish medicine and public health during the first third of the twentieth century, led the expedition. Other members were Luis Rodriguez Illera and Jorge Ramon Fananas, Cajal's son. Over four months they travelled through the Spanish territories of Guinea, collecting clinical and epidemiological information on sleeping sickness and other diseases and examining a great number of patients, including through haematological and parasitological measurements. In the clinical description of the 14 cases of trypanosomiasis studied we provided the first description of the opsoclonus-myoclonus syndrome. A pathological study of the brain was performed in one case. In addition, important entomological studies and experimental investigations on trypanosomiasis were performed. This expedition took place in the context of the desire to highlight again Spanish science headed by Cajal through the recently created Junta de Ampliacion de Estudios. In the investigations performed in Guinea, Pittaluga demonstrated a high scientific standard in the fields of clinical medicine, hygiene, parasitology and entomology, comparable with other contemporary European studies.

16096. **Mpanya, A., Hendrickx, D., Vuna, M., Kanyinda, A., Lumbala, C., Tshilombo, V., Mitashi, P., Luboya, O., Kande, V., Boelaert, M., Lefevre, P. & Lutumba, P., 2012.** Should I get screened for sleeping sickness? A qualitative study in Kasai province, Democratic Republic of Congo. *PLoS Neglected Tropical Diseases*, **6** (1): e1467.

Programme National de Lutte contre la Trypanosomiase Humaine Africaine, Kinshasa, Democratic Republic of Congo; Institute of Tropical Medicine, Antwerp, Belgium; Institut National de Recherche Biomédicale, Kinshasa, Democratic Republic of Congo; Bureau Diocesain des Œuvres Médicales, Mbuji-mayi, Democratic Republic of Congo; Université de Mbuji-mayi, Mbuji-mayi, Democratic Republic of Congo; Université de Kinshasa, Kinshasa, Democratic Republic of Congo; and Université de Lubumbashi, Lubumbashi, Democratic Republic of Congo. [dhendrickx@itg.be].

Control of human African trypanosomiasis (sleeping sickness) in the Democratic Republic of Congo is based on active mass population screening by mobile teams. Although generally

considered a successful strategy, the community participation rates in these screening activities and ensuing treatment remain low in the Kasai-Oriental province. A better understanding of the reasons behind this observation is necessary to improve regional control activities. Thirteen focus group discussions were held in five health zones of the Kasai-Oriental province to gain insights in the regional perceptions regarding sleeping sickness and the national control programme's activities. It was found that sleeping sickness is well known among the population and is considered a serious and life-threatening disease. The disease is acknowledged to have severe implications for the individual (e.g. persistence of manic periods and trembling hands, even after treatment), at the family level (e.g. income loss, conflicts, separations), and for communities (e.g. disruption of community life and activities). Several important barriers to screening and treatment were identified. Fear of drug toxicity, lack of confidentiality during screening procedures, financial barriers and a lack of communication between the mobile teams and local communities were described. Additionally, a number of regionally accepted prohibitions related to sleeping sickness treatment were described that were found to be a strong impediment to disease screening and treatment. These prohibitions, which do not seem to have a rational basis, have far-reaching socio-economic repercussions and severely restrict the participation in day-to-day life. It is concluded that a mobile screening calendar more adapted to the local conditions with more respect for privacy, the use of less toxic drugs, and a better understanding of the origin as well as better communication about the prohibitions related to treatment would facilitate higher participation rates among the Kasai-Oriental population in sleeping sickness screening and treatment activities organized by the national HAT control programme.

16097. **Mugasa, C. M., Adams, E. R., Boer, K. R., Dyserinck, H. C., Buscher, P., Schallig, H. D. & Leeflang, M. M., 2012.** Diagnostic accuracy of molecular amplification tests for human African trypanosomiasis: systematic review. *PLoS Neglected Tropical Diseases*, **6** (1): e1438.

Royal Tropical Institute, KIT Biomedical Research, Amsterdam, The Netherlands; Department of Veterinary Parasitology and Microbiology, Faculty of Veterinary Medicine, Makerere University Kampala, Kampala, Uganda; Amsterdam Institute of Global Health and Development, Amsterdam, The Netherlands; Academic Medical Centre Library, Academic Medical Centre, Amsterdam, The Netherlands; Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; and Department of Clinical Epidemiology, Biostatistics and Bioinformatics, Academic Medical Centre, Amsterdam, The Netherlands. [e.adams@kit.nl].

A range of molecular amplification techniques have been developed for the diagnosis of human African trypanosomiasis (HAT); however, careful evaluation of these tests must precede implementation to ensure their high clinical accuracy. Here, we investigated the diagnostic accuracy of molecular amplification tests for HAT, the quality of articles and reasons for variation in accuracy. Data from studies assessing diagnostic molecular amplification tests were extracted and pooled to calculate accuracy. Articles were included if they reported sensitivity and specificity or data whereby values could be calculated. Study quality was assessed using QUADAS and selected studies were analysed using the bivariate random effects model. Sixteen articles evaluating molecular amplification tests fulfilled the inclusion criteria: PCR (n = 12), NASBA (n = 2), LAMP (n = 1) and a study comparing PCR and NASBA (n = 1). Fourteen articles, including 19 different studies were included in the meta-analysis. Summary sensitivity for PCR on blood was 99.0 percent (95 percent CI 92.8 - 99.9) and the specificity was 97.7 percent (95 percent CI 93.0 - 99.3). Differences in study design and readout method did not significantly change estimates although use of satellite

DNA as a target significantly lowers specificity. Sensitivity and specificity of PCR on CSF for staging varied from 87.6 percent to 100 percent, and 55.6 percent to 82.9 percent respectively. It is concluded that PCR seems to have sufficient accuracy to replace microscopy where facilities allow, although this conclusion is based on multiple reference standards and a patient population that was not always representative. Future studies should, therefore, include patients for which PCR may become the test of choice and consider well designed diagnostic accuracy studies to provide extra evidence on the value of PCR in practice. Another use of PCR for control of disease could be to screen samples collected from rural areas, and test in reference laboratories to spot epidemics quickly and direct resources appropriately.

16098. **Oba, A., Gahtse, A., Ekouya Bowassa, G., Nika, E. & Obengui, 2011.** Congenital human African trypanosomiasis: an observation at the University Hospital of Brazzaville (Congo). *Archives de Pédiatrie*, **18** (10): 1114-1115.

Service de néonatologie, CHU de Brazzaville, BP 32, Brazzaville, Congo; Service de rhumatologie et de dermatologie, CHU de Brazzaville, BP 32, Brazzaville, Congo; and Service des maladies infectieuses, CHU de Brazzaville, BP 32, Brazzaville, Congo.[ekouyabg@yahoo.fr].

Abstract not available.

16099. **Ruiz-Postigo, J. A., Franco, J. R., Lado, M. & Simarro, P. P., 2012.** Human African trypanosomiasis in South Sudan: how can we prevent a new epidemic? *PLoS Neglected Tropical Diseases*, **6** (5): e1541.

World Health Organization, Regional Office for the Eastern Mediterranean, Cairo, Egypt; World Health Organization, Geneva, Switzerland; and Ministry of Health, Juba, Republic of South Sudan [postigoj@emro.who.int].

Human African trypanosomiasis (HAT) has been a major public health problem in South Sudan for the last century. Recurrent outbreaks with a repetitive pattern of responding-scaling down activities have been observed. Control measures for outbreak response were reduced when the prevalence decreased and/or socio-political crises erupted, leading to a new increase in the number of cases. This paper aims to raise international awareness of the threat of another outbreak of sleeping sickness in South Sudan. It is a review of the available data, interventions over time, and current reports on the status of HAT in South Sudan. Since 2006, control interventions and treatments providing services for sleeping sickness have been reduced. Access to HAT diagnosis and treatment has been considerably diminished. The current status of control activities for HAT in South Sudan could lead to a new outbreak of the disease unless 1) the remaining competent personnel are used to train younger staff to resume surveillance and treatment in the centres where HAT activities have stopped; and 2) control of HAT continues to be given priority even when the number of cases has been substantially reduced. Failure to implement an effective and sustainable system for HAT control and surveillance will increase the risk of a new epidemic. That would cause considerable suffering for the affected population and would be an impediment to the socio-economic development of South Sudan.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **35**: 16037, 16093]

16100. **Clerinx, J., Vlieghe, E., Asselman, V., Van de Casteele, S., Maes, M. B. & Lejon, V., 2012.** Human African trypanosomiasis in a Belgian traveller returning from the Masai Mara area, Kenya, February 2012. *Euro Surveillance*, **17** (10).

Department of Clinical Sciences, Institute of Tropical Medicine, Antwerp, Belgium. [jclerinx@itg.be].

A Belgian traveller was diagnosed with human African trypanosomiasis (HAT) due to *Trypanosoma brucei rhodesiense* nine days after visiting the Masai Mara area in Kenya. He presented with an inoculation chancre and was treated with suramin within four days of fever onset. Two weeks earlier, HAT was also reported in a German traveller who had visited the Masai Mara area. Because no cases have occurred in the area for over 12 years, this may indicate a focal cluster of HAT.

16101. **Cottle, L. E., Peters, J. R., Hall, A., Bailey, J. W., Noyes, H. A., Rimington, J. E., Beeching, N. J., Squire, S. B. & Beadsworth, M. B., 2012.** Multiorgan dysfunction caused by travel-associated African trypanosomiasis. *Emerging Infectious Diseases*, **18** (2): 287-289.

Royal Liverpool University Hospital, Liverpool, UK; Liverpool School of Tropical Medicine, Liverpool UK; University of Liverpool, Liverpool, UK; and Hawkshead Medical Practice, Ambleside, UK. [lucy.cottle@rlbuht.nhs.uk].

We describe a case of multiorgan dysfunction secondary to *Trypanosoma brucei rhodesiense* infection acquired on safari in Zambia. This case was one of several recently reported to ProMED-mail in persons who had travelled to this region. Trypanosomiasis remains rare in travellers but should be considered in febrile patients who have returned from trypanosomiasis-endemic areas of Africa.

16102. **Gobbi, F. & Bisoffi, Z., 2012.** Human African trypanosomiasis in travellers to Kenya. *Euro Surveillance*, **17** (10). Article 1.

Centro per le Malattie tropicali, Ospedale Sacro Cuore-Don Calabria, Negrar, Verona, Italy [federico.gobbi@sacrocuore.it].

The occurrence of two imported cases of *T. b. rhodesiense* HAT, who were returning from the Masai Mara area in south-west Kenya is not really surprising considering that several cases were reported in the last decade from the Serengeti Park in north-west Tanzania. Although located in two different countries, the two parks constitute a single geographical entity, artificially divided by the Kenyan–Tanzanian border. Up until now, transmission of the parasite occurred sporadically in the southern part (Serengeti) and seems to have now extended northward, probably following migration of infected game. The whole area should therefore be considered at potential risk. In 2001, the almost simultaneous occurrence of HAT in two Italian patients returning from Tarangire and Serengeti national parks was promptly reported to ProMED and to the European Network for Tropical Medicine and Travel Health (TropNet), allowing the detection of a cluster of several cases occurring in a short space of time in tourists who had been in the same locations. The importance of networks in Europe,

such as the European Travel Medicine Network (EuroTravNet) and TropNet, to detect rare diseases and to disseminate the relevant information, cannot be overemphasised. Besides offering advice on travel and prevention measures, such networks are also crucial for the local public health system in endemic countries, where tourism in the parks represents a fundamental income. For example, in 2001, after the alert was issued, surveillance of domestic cattle in the Serengeti and Tarangire areas was conducted by the chief veterinary officer in order to ascertain if they might have played a role in transmission of the parasite to humans. Awareness of HAT is an essential prerequisite to prompt diagnosis and disease management, thus avoiding the potentially fatal complications of the disease. For every patient coming from Sub-Saharan Africa, HAT, although rare, must be included in the differential diagnosis of any febrile patient returning from areas at potential risk. Patients often recall tsetse bites but this is not always the case as for example in the recent German case. Urech et al., in a review of the published cases, reported the presence of fever in the vast majority of cases of HAT due to *T. b. rhodesiense* (98 percent) and *T. b. gambiense* (93 percent). A trypanosomal chancre, which consists of a tender, purplish, indurated area that develops at the site of the tsetse fly bite, is a very important clue, occurring more frequently in *T. b. rhodesiense* disease (84 percent versus 47 percent in *T. b. gambiense* HAT). While its presence is virtually pathognomonic, its absence should not exclude the disease. Gastrointestinal and hepatic symptoms such as nausea, vomiting or jaundice are not rare in travellers infected with *T. b. rhodesiense* HAT and could mislead the physician to a gastrointestinal infection. In HAT patients, cardiac involvement with typical ECG alterations, as seen in the German case, is frequent. HAT cardiomyopathy generally subsides with treatment. Even in the absence of any accompanying symptoms, a fever in a patient coming from sub-Saharan Africa should prompt all clinicians to exclude malaria. If a thick blood smear is used for this, *T. b. rhodesiense* infection should not be missed, if present, as the sensitivity of a thick smear is high in the acute phase of the disease. However, in 11 percent of travellers infected with *T. b. rhodesiense*, trypanosomes could not be detected in the first blood smear and repeated blood examinations were necessary. An excessive reliance on malaria rapid diagnostic tests – which are increasingly suggested as a useful diagnostic tool, especially outside specialised, referral centres – might lead to *T. b. rhodesiense* HAT cases being missed, as well as other conditions such as relapsing fever caused by *Borrelia*. In the German case the reason why HAT was not diagnosed on presentation could be that malaria thin smear only was initially performed at the local hospital, which is a frequent practice in non-specialised centres, without doing the more sensitive thick smear. Whatever the reason, we argue that all travellers (including people who are long-term residents abroad and migrants) should have access to specialised (clinical and diagnostic) management if presenting with fever or other relevant symptoms. This is even more important for the *Gambiense* form of the disease, for which diagnosis is often more problematic. Moreover, while *T. b. rhodesiense* HAT cases have generally been tourists who have relatively easy access to appropriate healthcare, *T. b. gambiense* HAT outside endemic countries is typically observed in people who have been long-term residents overseas for missionary or work-related reasons or in migrants or refugees from endemic countries, including undocumented migrants who may have limited access to healthcare in the host country. Clinical networks such as TropNet, with its vast experience from its 62 centres spread over Europe, can also offer advice and support for diagnosis and management of HAT. As far as treatment is concerned, distribution of HAT drugs is the exclusive responsibility of the World Health Organization (WHO), as, except for pentamidine, they cannot be obtained on the market. To treat patients with imported HAT, hospital pharmacy services have to request drugs from WHO and provide patient data. The drugs are then received from WHO within 24 - 48 hours. However, to enable prompt start of treatment – which is particularly important for the acute *Rhodesiense* disease – a few hospitals have requested and have been given anti-trypanosomal

drugs and thus are repositories of these drugs. Ideally, at least one such repository should be present in every European country, in order to avoid unnecessary delay in drug procurement, which can also arise due to customs procedures. For some patients with first-stage *T. b. rhodesiense* HAT, treatment was initiated with the more readily available pentamidine, switching to suramin upon availability. As no vaccination is available, travellers to HAT-endemic areas should be alerted of this important albeit low risk and take general protective precautions. The tsetse fly is active during daytime and is particularly attracted by motion and blue and black surfaces. The patient reported in Germany used insect repellents, but wore shorts and short-sleeved shirts, while for the Belgian patient, this information is lacking. Bites can be prevented by wearing wrist- and ankle-length clothing of thick material and avoiding dark-coloured clothing. The fly is able to bite through thinly woven fabric: therefore the impregnation of clothing with permethrin is recommended, along with the application of a skin repellent. These measures should be particularly kept in mind now that transmission has recently occurred, and more cases might be expected. Moreover, all referral centres for imported tropical diseases should stay alert and any new case should be promptly reported to the concerned networks, as this would concur to a better knowledge of the local situation. The authors of the German paper report that the local authorities in Kenya have been duly informed and that a WHO team of experts has been sent to the area, therefore we hope to receive further information in the coming weeks.

16103. **Ilboudo, H., Berthier, D., Camara, M., Camara, O., Kabore, J., Leno, M., Keletigui, S., Chantal, I., Jamonneau, V., Belem, A. M., Cuny, G. & Bucheton, B., 2012.** APOL1 expression is induced by *Trypanosoma brucei gambiense* infection but is not associated with differential susceptibility to sleeping sickness. *Infection, Genetics & Evolution,* **12**(7): 1519-1523.

Centre International de Recherche-Développement sur l'Elevage en zone Subhumide (CIRDES), 01 BP 454 Bobo-Dioulasso 01, Burkina Faso; CIRAD, UMR INTERTRYP, Campus International de Baillarguet, F-34398 Montpellier, France; Programme National de Lutte contre la Trypanosomose Humaine Africaine, BP 851 Conakry, Guinea; Institut National de Santé Publique, Laboratoire National de Référence, BP 6366, Conakry, Guinea; Université Polytechnique de Bobo-Dioulasso, 01 BP 1091 Bobo-Dioulasso 01, Burkina Faso; and Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177, TA 207/G, Campus International de Baillarguet, F-34398 Montpellier Cedex 5, France. [bruno.bucheton@ird.fr].

Most African trypanosome species are sensitive to trypanolytic factors (TLFs) present in human serum. Trypanosome lysis was demonstrated to be associated with apolipoprotein L-I (APOL1). *Trypanosoma brucei* (*T. b.*) *gambiense* and *Trypanosoma brucei rhodesiense*, the two human infective trypanosome species, have both developed distinct resistance mechanisms to APOL1 mediated lysis. Whereas *T. b. rhodesiense* resistance is linked with the expression of the serum resistance associated (SRA) protein that interacts with APOL1 inside the parasite lysosome, inhibiting its lytic action; *T. b. gambiense* resistance is rather controlled by a reduced expression of the parasite HpHb receptor, limiting APOL1 absorption by trypanosomes. Based on this last observation we hypothesised that variation in the host APOL1 environment could significantly alter *T. b. gambiense* growth and thus resistance/susceptibility to sleeping sickness. To test this hypothesis, we have measured blood APOL1 relative expression in HAT patients, uninfected endemic controls and serologically positive subjects (SERO TL(+)) that are suspected to control infection to parasitological levels that are undetectable by the available test used in the field. All RNA samples were

obtained from medical surveys led in the HAT mangrove foci of Coastal Guinea. Results indicate that APOL1 expression is a complex trait dependant on a variety of factors that need to be taken into account in the analysis. Nevertheless, multivariate analysis showed that APOL1 expression levels were significantly higher in both HAT and SERO TL(+) subjects as compared with endemic controls (P=0.006). This result suggests that APOL1 expression is likely induced by *T. b. gambiense*, but is not related to resistance/susceptibility in its human host.

16104. **Jamonneau, V., Ilboudo, H., Kabore, J., Kaba, D., Koffi, M., Solano, P., Garcia, A., Courtin, D., Laveissiere, C., Lingue, K., Buscher, P. & Bucheton, B., 2012.** Untreated human infections by *Trypanosoma brucei gambiense* are not 100 percent fatal. *PLoS Neglected Tropical Diseases*, **6** (6): e1691.

Institut de Recherche pour le Développement, Unité Mixte de Recherche IRD-CIRAD 177, Campus International de Baillarguet, Montpellier, France; Centre International de Recherche-Développement sur l'Elevage en zones Subhumides (CIRDES), Unité de Recherches sur les Bases Biologiques de la Lutte Intégrée, Bobo-Dioulasso, Burkina Faso; Institut Pierre Richet, Unité de Recherche Trypanosomoses, Abidjan, Côte d'Ivoire; Université d'Abobo-Adjamé, URES de Daloa, Laboratoire de Génétique Moléculaire et Evolution des Maladies Infectieuses Tropicales, Daloa, Côte d'Ivoire; Institut de Recherche pour le Développement, Unité de Recherche 010, Faculté de Pharmacie, Paris, France; Programme National d'Elimination de la Trypanosomose Humaine Africaine, Abidjan, Côte d'Ivoire; and Institute of Tropical Medicine, Department of Biomedical Sciences, Antwerp, Belgium. [vincent.jamonneau@ird.fr].

The final outcome of infection by *Trypanosoma brucei gambiense,* the main agent of sleeping sickness, has always been considered as invariably fatal. While scarce and old reports have mentioned cases of self-cure in untreated patients, these studies suffered from the lack of accurate diagnostic tools available at that time. Here, using the most specific and sensitive tools available to date, we report on a long-term follow-up (15 years) of a cohort of 50 human African trypanosomiasis (HAT) patients from the Ivory Coast among whom 11 refused treatment after their initial diagnosis. In 10 out of 11 subjects who continued to refuse treatment despite repeated visits, parasite clearance was observed using both microscopy and the polymerase chain reaction (PCR). Most of these subjects (7/10) also displayed decreasing serological responses, becoming progressively negative to trypanosome variable antigens (LiTat 1.3, 1.5 and 1.6). Hence, in addition to the "classic" lethal outcome of HAT, we show that alternative natural progressions of HAT may occur: progression to an apparently aparasitaemic and asymptomatic infection associated with strong long-lasting serological responses and progression to an apparently spontaneous resolution of infection (with negative results in parasitological tests and PCR) associated with a progressive drop in antibody titres as observed in treated cases. While this study does not precisely estimate the frequency of the alternative courses for this infection, it is noteworthy that in the field national control programmes encounter a significant proportion of subjects displaying positive serologic test results but negative results in parasitological testing. These findings demonstrate that a number of these subjects display such infection courses. From our point of view, recognising that trypanotolerance exists in humans, as is now widely accepted for animals, is a major step forward for future research in the field of HAT.

16105. **Njamnshi, A. K., Seke Etet, P. F., Perrig, S., Acho, A., Funsah, J. Y., Mumba, D., Muyembe, J. J., Kristensson, K. & Bentivoglio, M., 2012.** Actigraphy in human African trypanosomiasis as a tool for objective clinical evaluation and monitoring: a

pilot study. *PLoS Neglected Tropical Diseases*, **6** (2): e1525.

Neurology Department, Central Hospital Yaoundé/Faculty of Medicine, University of Yaoundé I, Yaoundé, Cameroon. [alfred.njamnshi@gmail.com].

Human African trypanosomiasis (HAT) or sleeping sickness leads to a complex neuropsychiatric syndrome with characteristic sleep alterations. Current division into a first, haemolymphatic stage and second, meningoencephalitic stage is primarily based on the detection of white blood cells and/or trypanosomes in the cerebrospinal fluid. The validity of this criterion is, however, debated, and novel laboratory biomarkers are under study. Objective clinical HAT evaluation and monitoring is therefore needed. Polysomnography has effectively documented sleep-wake disturbances during HAT, but could be difficult to apply as routine technology in field work. The non-invasive, cost-effective technique of actigraphy has been widely validated as a tool for the ambulatory evaluation of sleep disturbances. In this pilot study, actigraphy was applied to the clinical assessment of HAT patients. In this study, actigraphy was recorded in patients infected by *Trypanosoma brucei gambiense*, and age- and sex-matched control subjects. Simultaneous nocturnal polysomnography was also performed in the patients. Nine patients, including one child, were analysed at admission and two of them also during specific treatment. Parameters, analysed with user-friendly software, included sleep time evaluated from rest-activity signals, rest-activity rhythm waveform and characteristics. The findings showed sleep-wake alterations of various degrees of severity, which in some patients did not parallel white blood cell counts in the cerebrospinal fluid. Actigraphic recording also showed improvement of the analysed parameters after treatment initiation. Nocturnal polysomnography showed alterations of sleep time closely corresponding to those derived from actigraphy. The data indicate that actigraphy can be an interesting tool for HAT evaluation, providing valuable clinical information through simple technology, well suited also for long-term follow-up. Actigraphy could therefore objectively contribute to the clinical assessment of HAT patients. This method could be incorporated into a clinical scoring system adapted to HAT to be used in the evaluation of novel treatments and laboratory biomarkers.

16106. **Pakasa, N. M. & Sumaili, E. K., 2012.** Pathological peculiarities of chronic kidney disease in patient from sub-Saharan Africa. Review of data from the Democratic Republic of Congo. *Annales de Pathologie*, **32** (1): 40-52.

Service d'anatomie pathologique, cliniques universitaires de Kinshasa, Université de Kinshasa, BP 864, Kinshasa XI, Democratic Republic of Congo (DRC). [nmpakasa@free.fr].

Chronic kidney disease (CKD) is a major global public health problem. But kidney involvement is more common and appears more severe in Africa than in developed countries. The likely causes of end stage renal disease (ESRD) or CKD stage 3 and above in developed countries are diabetes, hypertension and less frequently glomerular diseases. In contrast, in decreasing order in Africa are glomerulopathies, hypertension and diabetes. The reasons for this preponderance of glomerular diseases are not fully known but may be linked to the persistence or re-emergence of tropical diseases. This study reviews the kidney involvements more associated with common tropical diseases including HIV/AIDS. The most common HIV/AIDS lesion is a specific focal and segmental glomerulosclerosis (FSGS) termed HIV-associated nephropathy (HIV-AN). Renal complications of tropical parasites are heterogeneous. Various glomerulopathies like FSGS occur during various filariasis infections. *Schistosoma mansoni* is responsible for membranoproliferative glomerulonephritis and

amyloidosis. Human African trypanosomiasis is associated with cryoglobulinaemic membranoproliferative glomerulonephritis. The *Plasmodium malariae* is mainly responsible for membranoproliferative glomerulonephritis. Acute patterns (acute tubular necrosis or acute postinfectious glomerulonephritis) are observed during *Plasmodium falciparum* infection. Several other viral, bacterial or mycobacterial infections like leprosy and tuberculosis still prevalent in Africa can also affect the kidney. Sickle cell disease is responsible for a variety of renal injuries. In conclusion, kidney lesions linked to tropical diseases partly explain the peculiar pattern of CKD of the black race and play a significant role in the current outbreak of the CKD in sub Saharan Africa.

16107. **Richter, J., Gobels, S., Gobel, T., Westenfeld, R., Muller-Stover, I. & Haussinger, D., 2012.** A returning traveller with fever, facial swelling and skin lesions. *BMJ*, **344**: e2092.

University Hospital for Gastroenterology, Hepatology and Infectious Diseases, Heinrich-Heine-University, D-40225 Dusseldorf, Germany. [joachim.richter@med.uni-duesseldorf.de].

No abstract available.

16108. **Truc, P., Lando, A., Penchenier, L., Vatunga, G. & Josenando, T., 2012.** Human African trypanosomiasis in Angola: clinical observations, treatment, and use of PCR for stage determination of early stage of the disease. *Transactions of the Royal Society of Tropical Medicine & Hygiene*, **106** (1): 10-14.

Institut de Recherche pour le Développement, Unité Mixte de Recherche 177 IRD-CIRAD, Campus International de Baillarguet, TA A17/G, 34398, Montpellier Cedex 5, France. [Philippe.Truc@ird.fr].

Biological and clinical observations are described for 224 patients infected by human African trypanosomiasis (HAT) in Angola in 2007 and 2008. Seven patients were initially classified in stage 1 (S1), 17 intermediate stage (IS) (WBC <20 lymphocytes/µL with absence of trypanosomes in cerebrospinal fluid (CSF) and no neurological signs), and 200 in stage 2 (S2). Out of 224 patients, 165 (73.6 percent) presented one or more neurological signs. During treatment with eflornithine, six deaths of S2 patients occurred, five of which were because of an encephalopathy syndrome. Nine patients were diagnosed with a relapse or suspected treatment failure during the follow-up: eight patients after treatment with eflornithine (relapse rate 4.1 percent) and one patient after pentamidine (6.6 percent). The contribution of PCR for stage determination evaluated for S1 and IS confirms the difficulty of stage determination, as one S1 patient and two IS patients were carriers of trypanosomes detected *a posteriori* by PCR in CSF but were treated with pentamidine while follow-up did not confirm treatment efficacy. Since 2001 in Angola, either by passive or active detection mode, approximately 80 percent of the new cases every year were in S2, whereas the annual number of cases has regressed, probably because the transmission of HAT is decreasing. However, stage determination and treatment remain two major issues for managing the chronic form of sleeping sickness.

16109. **Truc, P., Tiouchichine, M. L., Cuny, G., Vatunga, G., Josenando, T., Simo, G. & Herder, S., 2012.** Multiple infections of *Trypanosoma brucei gambiense* in blood and cerebrospinal fluid of human African trypanosomosis patients from Angola: consequences on clinical course and treatment outcome. *Infection, Genetics &*

Evolution, **12** (2): 399-402.

Institut de Recherche pour le Développement, Unité Mixte de Recherche 177 IRD-CIRAD, Campus International de Baillarguet, TA A17/G, 34398 Montpellier Cedex 5, France; Instituto de Combate e Controlo das Tripanossomiases, Ministério da Saúde, 168 rua Cdte Kwehna, Ingombota, CP 2657 Luanda, Angola; Department of Biochemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon; and Institut de Recherche pour le Développement, Unité Mixte de Recherche 177 IRD-CIRAD, Kasetsark University Chatuchak, Bangkok, Thailand. [Philippe.Truc@ird.fr, truc@ird.fr].

Human African trypanosomosis, caused by *Trypanosoma brucei gambiense*, is a chronic disease, although various clinical patterns have been observed, from asymptomatic to acute forms. Since 2001 in Angola, 80 percent of patients have been found to be in the meningoencephalitic stage of the disease. The existence of an acute form of the disease caused by virulent strains of trypanosomes was suspected. To test this hypothesis, four sensitive and polymorphic microsatellite markers were used to characterize the trypanosome DNA extracted from the blood and cerebrospinal fluid of 100 patients in the meningoencephalitic stage. Twenty-three patients were found with mixed *T. b. gambiense* genotypes in the blood and/or cerebrospinal fluid. The absence of association between the number of infecting genotypes, the presence of neurological signs and white blood cell counts in the cerebrospinal fluid, seems to indicate, at least in the context of the present study, the absence of virulent strains. However, out of five patients who died from encephalopathy syndrome during treatment with eflornithine, three harboured multiple infections.

16110. **Wolf, T., Wichelhaus, T., Gottig, S., Kleine, C., Brodt, H. R. & Just-Nuebling, G., 2012.** *Trypanosoma brucei rhodesiense* infection in a German traveller returning from the Masai Mara area, Kenya, January 2012. *Euro Surveillance*, **1**7 (10).

Department of Internal Medicine 2 - Infectious Diseases, Hospital of the J. W. Goethe University, Frankfurt, Germany. [timo.wolf@kgu.de].

In January 2012, a case of human African Trypanosomiasis (HAT) was identified in Germany in a traveller returning from the Masai Mara area in Kenya. The 62-year-old man had travelled to the Masai Mara game park from 18 - 19 January 2012 and developed fever on 28 January. The infection with *Trypanosoma brucei rhodesiense* was confirmed by laboratory testing three days hereafter.

(c) TREATMENT

[See also **35**: 16047, 16056, 16108, 16109]

16111. **Gradmann, C., 2011.** Magic bullets and moving targets: antibiotic resistance and experimental chemotherapy, 1900-1940. *Dynamis*, **31** (2): 305-321.

Section for Medical Anthropology and Medical History, University of Oslo, Norway. [christoph.gradmann@medisin.uio.no].

It was in the 1940s that antibiotic resistance arose as an object of study for clinical medicine. Somewhat earlier it had become an important analytical tool for bacterial geneticists. However, the concept of antibiotic resistance as an induced and inheritable trait of

microbial species was introduced a generation earlier in the years preceding the First World War. This paper reconstructs the concept that was put forward by the German immunologist Paul Ehrlich in 1907. He came across the phenomenon when trying to develop chemotherapies for trypanosomiasis, the best known of which is African sleeping sickness. However, resistance was studied by him for other than therapy-related purposes. It provided a productive laboratory model for the study of cell functions. Induced resistance to chemicals facilitated the development of ideas on the relation of a parasite's cellular metabolism and of drug action, i.e. by providing a negative proof for the existence of chemoreceptors on the surfaces of parasite cells. This approach also serves to explain why British and German researchers continued to study the phenomenon of induced resistance in microbes for decades -despite it being absent from clinical medicine. After all, very few chemotherapies of infectious diseases existed prior to the arrival of the sulpha drugs. Moreover, resistance to such medicines was rarely observed. However, being part and parcel of Ehrlich's theories, his views on resistance were also criticised together with these. It was in particular Henry Dale who would challenge Ehrlich's views of resistance being an inheritable and stable trait of microbes. Instead he insisted that understanding this "wholly mysterious phenomenon" required taking into account some host interaction. Induced resistance, which had come into being as a chance discovery aboutthe chemotherapy of sleeping sickness, thus became one of the more important laboratory models of twentieth-century immunological research. Its early history is largely discontinuous with later work, and antimicrobial resistance as it evolved from 1900 to 1940 followed other trajectories than those which became relevant after 1940.

16112. **Priotto, G., Chappuis, F., Bastard, M., Flevaud, L. & Etard, J. F., 2012.** Early prediction of treatment efficacy in second-stage *Gambiense* human African trypanosomiasis. *PLoS Neglected Tropical Diseases*, **6** (6): e1662.

Epicentre, Paris, France; Operational Centre Geneva, Médecins sans Frontières, Geneva University Hospitals, Geneva, Switzerland; Operational Centre Barcelona-Athens, Médecins sans Frontières, Barcelona, Spain; and Institut de Recherche pour le Développement/UMI 233, Montpellier, France. [gpriotto@neuf.fr].

Human African trypanosomiasis is fatal without treatment. The long post-treatment follow-up (24 months) required to assess cure complicates patient management and is a major obstacle in the development of new therapies. We analysed individual patient data from 12 programmes conducted by Médecins Sans Frontières in Uganda, Sudan, Angola, Central African Republic, Republic of Congo and Democratic Republic of Congo searching for early efficacy indicators. Patients analysed had confirmed second-stage disease with complete follow-up and confirmed outcome (cure or relapse), and had CSF leucocytes counts (CSFLC) performed at six months post-treatment. We excluded patients with uncertain efficacy outcomes: incomplete follow-up, death, relapse diagnosed with CSFLC below 50/µL and no trypanosomes. We analysed the 6-month CSFLC via receiver-operator-characteristic curves. For each cut-off value we calculated sensitivity, specificity and likelihood ratios (LR+ and LR-). We assessed the association of the optimal cut-off with the probability of relapsing via random-intercept logistic regression. We also explored two-step (6 and 12 months) composite algorithms using the CSFLC. The most accurate cut-off to predict outcome was 10 leucocytes/µL (n = 1 822, 76.2 percent sensitivity, 80.4 percent specificity, 3.89 LR+, 0.29 LR-). Multivariate analysis confirmed its association with outcome (odds ratio = 17.2). The best algorithm established cure at six months with < = 5 leucocytes/µL and relapse with > = 50 leucocytes/µL; patients between these values were discriminated at 12 months by a 20 leucocytes/µL cut-off (n = 2 190, 87.4 percent sensitivity, 97.7 percent specificity, 37.84 LR+, 0.13 LR-). It is concluded that the six-month CSFLC can predict outcome with some

limitations. Two-step algorithms enhance the accuracy but impose 12-month follow-up for some patients. For early estimation of efficacy in clinical trials and for individual patients in the field, several options exist that can be used according to priorities.

16113. **Simarro, P. P., Franco, J., Diarra, A., Postigo, J. A. & Jannin, J., 2012.** Update on field use of the available drugs for the chemotherapy of human African trypanosomiasis. *Parasitology*, **139** (7): 842-846.

World Health Organization, HTM/NTD 20, avenue Appia 1211, Geneva 27, Switzerland. [simarrop@who.int].

Despite the fact that eflornithine was considered as the safer drug to treat human African trypanosomiasis (HAT) and has been freely available since 2001, the difficulties in logistics and cost burden associated with this drug meant that the toxic melarsoprol remained the drug of choice. The World Health Organization responded to the situation by designing a medical kit containing all the materials needed to use eflornithine, and by implementing a training and drugs distribution programme which have allowed a transition to this much safer treatment. The introduction of the combination of nifurtimox and eflornithine (NECT) has accelerated the shift from melarsoprol to the best treatment available, due to reduced dosage and treatment time for eflornithine that has significantly lessened the cost and the burden of logistics encountered during treatment and distribution. The decrease in the use of more dangerous but cheaper melarsoprol has meant a rise in the cost per patient of treating HAT. Although NECT is cheaper than eflornithine monotherapy, an unexpected consequence has been a continuing rise in the cost per patient of treating HAT. The ethical decision of shifting to the best available treatment imposes a financial burden on HAT control programmes that might render long-term application unsustainable. These factors call for continuing research to provide new safer and more effective drugs that are simple to administer and cheaper when compared with current drugs.

6. ANIMAL TRYPANOSOMOSIS

(a) SURVEY AND DISTRIBUTION

[See also **35**: 16091]

16114. **Berlin, D., Nasereddin, A., Azmi, K., Ereqat, S., Abdeen, Z., Eyal, O. & Baneth, G., 2012.** Prevalence of *Trypanosoma evansi* in horses in Israel evaluated by serology and reverse dot blot. *Research in Veterinary Science*. **Available online 11 May**.

Veterinary Teaching Hospital, Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel; Al-Quds Nutrition and Health Research Center, Faculty of Medicine, Al-Quds University, Abu-Deis, P.O. Box 20760, West Bank, Palestine; and Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, P.O. Box 12, Rehovot 76100, Israel. [berlin@agri.huji.ac.il].

Trypanosoma evansi is the cause of surra in horses, camels and other domestic animals. Following the first outbreak of surra in horses and camels in Israel in 2006, a survey of the prevalence of the parasite in the Israeli horse population was conducted using serology, PCR followed by the reverse dot blot (RDB) technique and blood smear microscopy. In total, 614

horses from seven regions were sampled. The CATT/*T. evansi* kit was used for serology for all the horses. Horses from the Arava and Dead Sea region, where the first outbreak occurred, were sampled again one year later and both samples were subjected to serology and the RDB technique. The country wide seroprevalence was 4.6 percent (28/614). The seroprevalence in the Arava and Dead Sea region was 6.5 percent (9/139) in the first sampling compared with 4.1 percent (5/122) in the second, whereas the prevalence of RDB-positivity was 18.7 percent (26/139) in the first sampling and only 0.8 percent (1/122) in the second. All horses were asymptomatic except for one horse from the Arava and Dead Sea region that demonstrated clinical signs of surra combined with positive serology and RDB. The results of this study indicate that surra is prevalent in most regions of the country and thus should be considered for differential diagnosis in horses and other domestic animals in Israel with chronic weight loss, oedema or neurological signs.

16115. **Moti, Y., Fikru, R., Van Den Abbeele, J., Buscher, P., Van den Bossche, P., Duchateau, L. & Delespaux, V., 2012.** Ghibe river basin in Ethiopia: present situation of trypanocidal drug resistance in *Trypanosoma congolense* using tests in mice and PCR-RFLP. *Veterinary Parasitology*. **Available online 26 April.**

Department of Microbiology and Veterinary Public Health, Jimma University, College of Agriculture and Veterinary Medicine, P.O. Box 307, Jimma, Ethiopia; School of Veterinary Medicine, Addis Ababa University, Debre Zeit 34, Ethiopia; Institute of Tropical Medicine, Department of Biomedical Sciences - Parasitology - Veterinary Protozoology, Nationalestraat 155, Antwerp, Belgium; Institute of Tropical Medicine, Animal Health Department, Disease Control Unit, Nationalestraat 155, Antwerp, Belgium; University of Pretoria, Private Bag X04, Onderstepoort 0110, South Africa; and Universiteit Gent - Faculty of Veterinary Sc. - Physiology, & Biometry, Salisburylaan 133, B-9820 Merelbeke, Belgium. [vdelespaux@itg.be].

A cross-sectional study was carried out in the Ghibe valley from August to October 2010. 411 head of cattle were sampled in eight villages for buffy coat examination (BCE) and blood spots were collected from each animal for trypanosomosis diagnosis by 18S-PCR-RFLP and diminazene aceturate (DA) resistance by Ade2-PCR-RFLP. Three villages were selected in a zone where trypanosomosis control operations are currently on-going, whereas the other five villages were located outside these control operations. Twenty-four samples (5.84 percent) were diagnosed positive for *Trypanosoma congolense* by BCE and injected in mice for further characterization. Twelve of those isolates successfully multiplied in mice and were tested by an *in vivo* mouse test for diminazene (DA) (10 and 20 mg/kg bw.) and isometamidium (ISM) (1mg/kg bw) resistance. All were shown to be resistant to both drugs at all doses. The use of the Ade2-PCR-RFLP on these isolates confirmed their DA-resistance profile. Seventy-three of the collected blood spots (17.8 percent) were diagnosed positive for *T. congolense* by 18S-PCR-RFLP of which 37 (50.7 percent) gave amplification products with the Ade2-PCR-RFLP. Here, 35 (94.6 percent) showed a resistant profile, one (2.7 percent) a sensitive profile and one (2.7 percent) a mixed profile. The data were analysed by a logistic regression model and the relapsing time in mice tests was assessed using the Cox regression model. There was no significant intervention effect ($p=0.83$) with odds ratio equal to 1.21 when using the BCE data. The 18S-PCR-RFLP test also showed no significant intervention effect ($p=0.60$) with odds ratio equal to 1.43. The hazard ratio of getting parasitaemic after treatment with DA at 20mg/kg bwcompared with the control group was 0.38 which differs significantly from one ($p<0.001$). Relapsing time after treatment with DA 10mg/kg bw or ISM 1mg/kg bwwas also significantly longer than the prepatent period of the control group. The situation of drug resistance in the Ghibe valley is further discussed.

16116. **Rahman, W. A., Fong, S., Chandrawathani, P., Nurulaini, R., Zaini, C. M. & Premalaatha, B., 2012.** Comparative seroprevalences of bovine trypanosomiasis and anaplasmosis in five states of Malaysia. *Tropical Biomedicine*, **29** (1): 65-70.

School of Biological Sciences, Universiti Sains Malaysia, Minden, 11800 Penang, Malaysia.

A comparative seroprevalence study on bovine trypanosomiasis and anaplasmosis was conducted. Sera of adult cattle and buffaloes of different breeds from farms from five different states in Malaysia were collected and tested for the presence of *Trypanosoma evansi* antibodies by CATT and *Anaplasma marginale* antibodies by c-ELISA. Of the 116 samples, 14.7 percent tested positive for bovine trypanosomiasis and 77.6 percent for bovine anaplasmosis.

(b) PATHOLOGY AND IMMUNOLOGY

16117. **Tesfaye, D., Speybroeck, N., De Deken, R. & Thys, E., 2012.** Economic burden of bovine trypanosomosis in three villages of Metekel zone, northwest Ethiopia. *Tropical Animal Health & Production*, **44** (4): 873-879.

School of Veterinary Medicine, Hawassa University, P.O. Box 5, Hawassa, Ethiopia. [dawit89@yahoo.com].

The study was carried out to assess the economic burden of trypanosomosis in three villages of the Metekel zone in 2009. The disease was found to cause substantial economic losses through cattle mortality, drug purchase, and draft power loss of infected oxen. The farmers in the area were spending a significantly ($P < 0.05$) higher amount of money for the treatment of trypanosomosis than all other diseases combined. The overall mortality rate of cattle due to trypanosomosis was 4.4 percent. The mortality was significantly higher ($P < 0.05$) in an area where trypanosomosis prevalence was also higher. Many of the farmers prioritized losses of draft power as the most important impact of the disease. The overall prevalence of the disease was 12.1 percent. The disease burden was significantly ($P < 0.05$) higher in the rainy season than at other times of the year. In general, farmers had good knowledge on the signs and seasonality of trypanosomosis. Thus, tsetse suppression activities that involve the local community can be an important tool towards minimizing the economic burden of the disease in the area.

(c) TRYPANOTOLERANCE

[See also **35**: 16060]

16118. **Dayo, G. K., Gautier, M., Berthier, D., Poivey, J. P., Sidibe, I., Bengaly, Z., Eggen, A., Boichard, D. & Thevenon, S., 2012.** Association studies in QTL regions linked to bovine trypanotolerance in a West African crossbred population. *Animal Genetics*, **43** (2): 123-132.

IRD, UMR 177, Montpellier, France; CIRDES, BP454 Bobo Dioulasso, Burkina Faso; INRA, UMR1031 CBGP, Montferrier-sur-Lez F-34988, France; CIRAD, UMR

Trypanosomes, F-34398 Montpellier, France; CIRAD, UPR Systèmes d'élevage, F-34398 Montpellier, France; INRA, UR631 Station d'Amélioration Génétique des Animaux, 31326 Castanet-Tolosan, France; INRA, UMR 1313 Génétique Animale et Biologie Intégrative, 78352 Jouy-En-Josas, France; and Illumina, UK Ltd, Essex, UK. [sophie.thevenon@cirad.fr].

African animal trypanosomosis is a parasitic blood disease transmitted by tsetse flies and is widespread in sub-Saharan Africa. West African taurine breeds have the ability, known as trypanotolerance, to limit parasitaemia and anaemia and remain productive in enzootic areas. Several quantitative trait loci (QTL) underlying traits related to trypanotolerance have been identified in an experimentally infected F_2 population resulting from a cross between taurine and Zebu cattle. Although this information is highly valuable, the QTL remain to be confirmed in populations subjected to natural conditions of infection, and the corresponding regions need to be refined. In our study, 360 West African cattle were phenotyped for the packed cell volume control under natural conditions of infection in south-western Burkina Faso. Phenotypes were assessed by analysing data from previous cattle monitored over two years in an area enzootic for trypanosomosis. We further genotyped for 64 microsatellite markers mapping within four previously reported QTL on BTA02, BTA04, BTA07 and BTA13. These data enabled us to estimate the heritability of the phenotype using the kinship matrix between individuals computed from genotyping data. Thus, depending on the estimators considered and the method used, the heritability of anaemia control ranged from 0.09 to 0.22. Finally, an analysis of association identified an allele of the MNB42 marker on BTA04 as being strongly associated with anaemia control, and a candidate gene, INHBA, as being close to that marker.

16119. **Desta, T. T., Ayalew, W. & Hegde, P. B., 2012.** Farmers' perceptions on trypanosomosis and trypanotolerance character of the taurine Sheko. *Tropical Animal Health & Production*, **44** (3): 609-616.

Wolaita Sodo Agricultural Technical Vocational Education and Training College, P.O. Box 120, Wolaita Sodo, Ethiopia. [takele_taye@yahoo.com].

In the humid and subhumid tropics, trypanosomosis is an economically important zoonotic protozoan disease of the commonly kept farm animal species and their wild relatives. For example, more than 20 percent of the humid western and southwestern Ethiopia, which is home to more than 14 million heads of cattle, is under varying levels of trypanosomosis risk. Our study was therefore initiated to document farmers' perception on trypanosomosis and Sheko's trypanotolerance character. Our findings showed that trypanosomosis was the most frequently reported cattle disease in the Bench Maji Zone. Accordingly, 76.7 percent of the farmers reported the epidemiological importance of trypanosomosis, and they also noted that trypanosomosis on average accounted for 63.0 percent of annualized cattle death. The reported signs of trypanosomosis and trypanotolerance indicators were consistent with literature reports. Moreover, 66.7 percent of the farmers reported Sheko's trypanotolerance character. In the course of time, smallholder farmers have developed ethnoveterinary practices that are mainly used to prevent the landing of vector flies on the animal. Wet and warm seasons of the year, i.e. spring and to some extent the beginning of summer and autumn, were reported as peak periods of trypanosomosis risk. Therefore, this shows the need for incorporating farmers' knowledge in trypanosomosis control programmes.

16120. **Flori, L., Gonzatti, M. I., Thevenon, S., Chantal, I., Pinto, J., Berthier, D., Aso, P. M. & Gautier, M., 2012.** A quasi-exclusive European ancestry in the Senepol tropical

cattle breed highlights the importance of the slick locus in tropical adaptation. *PLoS One*, **7** (5): e36133.

INRA, UMR 1313 GABI, F-78350 Jouy-en-Josas, France; CIRAD, UMR INTERTRYP, Montpellier, France; Departamento de Biología Celular, Universidad Simón Bolívar, Caracas, Venezuela; and INRA, UMR CBGP, Montferrier-sur-Lez, France. [laurence.flori@jouy.inra.fr].

The Senepol cattle breed (SEN) was created in the early 20th century from a presumed cross between a European (EUT) breed (Red Poll) and a West African taurine (AFT) breed (N'Dama). Well adapted to tropical conditions, it is also believed to be trypanotolerant according to its putative AFT ancestry. However, such origins needed to be verified to define relevant husbandry practices and the genetic background underlying such adaptation needed to be characterized. We genotyped 153 SEN individuals on 47 365 SNPs and combined the resulting data with those available on 18 other populations representative of EUT, AFT and Zebu (ZEB) cattle. We found on average 89 percent EUT, 10.4 percent ZEB and 0.6 percent AFT ancestries in the SEN genome. We further looked for footprints of recent selection using standard tests based on the extent of haplotype homozygosity. We underlined i) three footprints on chromosome (BTA) 01, two of which are within or close to the polled locus underlying the absence of horns, and ii) one footprint on BTA20 within the slick hair coat locus, involved in thermotolerance. Annotation of these regions allowed us to propose three candidate genes to explain the observed signals (TIAM1, GRIK1 and RAI14). Our results do not support the accepted concept about the AFT origin of the SEN breed. Initial AFT ancestry (if any) might have been counter-selected in early generations due to breeding objectives oriented in particular toward meat production and hornless phenotype. Therefore, SEN animals are likely susceptible to African trypanosomes which questions the importation of SEN within the West African tsetse belt, as promoted by some breeding societies. Besides, our results revealed that the SEN breed is predominantly a EUT breed well adapted to tropical conditions and confirmed the importance in thermotolerance of the slick locus.

(d) TREATMENT

[See **35**: 16079, 16080, 16085, 16115]

7. EXPERIMENTAL TRYPANOSOMOSIS

(a) DIAGNOSTICS

[See also **35**: 16097]

16121. **Bieler, S., Matovu, E., Mitashi, P., Ssewannyana, E., Bi Shamamba, S. K., Bessell, P. R. & Ndung'u, J. M., 2012.** Improved detection of *Trypanosoma brucei* by lysis of red blood cells, concentration and LED fluorescence microscopy. *Acta Tropica*, **121** (2): 135-140.

Foundation for Innovative New Diagnostics, Geneva, Switzerland. [sylvain.bieler@finddiagnostics.org].

Confirmatory diagnosis of African trypanosomiasis relies on demonstration of parasites in body fluids by bright field microscopy. The parasitaemia in infected patients and animals is

usually low, and concentration methods are used to try and increase the chances of seeing parasites. Recently, fluorescence microscopes using light-emitting diodes (LED) have been developed. Since they emit strong light, their use does not require a dark room, making field application a possibility. We have combined LED fluorescence microscopy with lysis of red blood cells (RBC) to improve the sensitivity and speed of detecting trypanosomes. In studies conducted at four centres in Uganda and the Democratic Republic of the Congo, parasitaemic blood was serially diluted and the RBCs lysed using commercial buffer. Samples were then concentrated by centrifugation, and different volumes of the sediment used to make thin and thick smears. Next, these were stained with acridine orange or Giemsa, and examined using an LED microscope under fluorescence or bright light, respectively. Detection of parasites was significantly improved by RBC lysis and concentration, regardless of the staining and microscopy method used. Further improvements were made when smears were prepared using larger volumes of sediment. The best results were obtained with thin smears prepared using 20 µL of sediment and stained with acridine orange. The time taken to see the first parasite was dramatically reduced when smears were examined by LED fluorescence microscopy, compared with bright light. LED fluorescence microscopy was found to be easier and required less visual effort than bright field microscopy. These studies demonstrate the potential for incremental improvement in detection of *Trypanosoma brucei* by combining LED fluorescence microscopy with RBC lysis and concentration. The lysis and concentration method may also be useful in sample preparation for other diagnostic tests for trypanosomiasis.

16122. **Franco, J. R., Simarro, P. P., Diarra, A., Ruiz-Postigo, J. A. & Jannin, J. G., 2012.** The human African trypanosomiasis specimen biobank: a necessary tool to support research of new diagnostics. *PLoS Neglected Tropical Diseases*, **6** (6): e1571.

World Health Organization, Control of Neglected Tropical Diseases, Innovative and Intensified Disease Management, Geneva, Switzerland; World Health Organization, Regional Office for Africa, Brazzaville, Congo; and World Health Organization, Regional Office for the Eastern Mediterranean, Cairo, Egypt. [francoj@who.int].

Human African trypanosomiasis (HAT), or sleeping sickness, is a vector-borne disease caused by trypanosomes (*Trypanosoma brucei gambiense* and *T. b. rhodesiense*) mainly affecting impoverished rural areas in sub-Saharan Africa, where the health systems are weak. Over the last decade, the number of HAT cases has shown a decreasing trend as a result of coordinated control efforts. This makes it possible to envisage the elimination of the disease, but a new approach to uphold current results is needed. Sustainability of the control efforts will require integration of control and surveillance activities within a reinforced health system. However, the complexity of the existing diagnostic tools is not compatible with prevailing conditions at basic health facilities in rural areas where the disease is endemic, which hinders the participation of the health system in the control and surveillance of the disease. There is an urgent need for diagnostic tests that are reliable, cheap, and easy to perform at basic health services. In 2006, the Department of Control of Neglected Tropical Diseases (NTD) of the World Health Organization (WHO) established collaboration with the Foundation for Innovative New Diagnostics FIND, to develop new diagnostic tools for the control of HAT that meet the requirements of a sustainable elimination approach. In the framework of this agreement, WHO established a HAT specimen biobank as a collection of biological specimens related to HAT, coupled with clinical and epidemiological information of the person who donated the specimens. The specimen biobank is the property of WHO and its main objective is to provide clinical reference material to research institutions to facilitate the development and evaluation of new tests for the diagnosis of HAT. To set up a specimen

bank for HAT first requires the collection of specimens while strictly following good clinical practice principles. The specimens have to be collected in the areas where the disease is endemic, usually remote areas with limited health resources and impoverished affected populations. The specimens collected have to be well identified and kept in strict cold chain from the time of collection to the final storage. To fulfil these conditions is challenging, but we have proved that it is not insurmountable.

16123. **Goto, Y., Duthie, M. S., Nguyen, T. T., Asada, M., Kawazu, S., Carter, D. & Inoue, N., 2011.** Serological characterizations of tandem repeat proteins for detection of African trypanosome infection in cattle. *Parasitology International*, **60** (4): 538-540.

National Research Center for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan; Laboratory of Molecular Immunology, Graduate School of Agricultural and Life Sciences, The University of Tokyo, Tokyo, Japan; Protein Advances Inc., Seattle, USA; and Infectious Disease Research Institute, Seattle, USA. [aygoto@mail.ecc.u-tokyo.ac.jp].

Serological diagnosis is a useful method to detect African trypanosome infection in livestock. Currently available serological tests utilize whole parasites or crude antigens, and recombinant antigens may improve reproducibility/standardization and reduce production costs. With a goal of identifying such recombinant proteins, we computationally identified proteins with tandem repeat (TR) domain from the parasite proteomes and evaluated their potential for serological diagnosis of African trypanosome infections in cattle. Among those tested, Tbg4 demonstrated the best performance with 92 percent sensitivity, followed by TbbGM6 (85 percent), TcoGM6 (85 percent), Tbg2 (65 percent) and Tbg5 (65 percent). Although further evaluations such as investigating cross-reactivity to other infections are needed, our data indicate the potential of these antigens for detection of African trypanosome infection in cattle.

16124. **Milocco, C., Kamyingkird, K., Desquesnes, M., Jittapalapong, S., Herbreteau, V., Chaval, Y., Douangboupha, B. & Morand, S., 2012.** Molecular demonstration of *Trypanosoma evansi* and *Trypanosoma lewisi* DNA in wild rodents from Cambodia, Lao PDR and Thailand. *Transboundary & Emerging Diseases*. **Published online 9 February.**

Centre de Coopération Internationale en Recherche Agronomique pour le Développement, Montpellier, France; Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Chatuchak, Bangkok Thailand; Institut des Sciences de l'Evolution, UMR 5554 CNRS-IRD-UM2, CC65, Université de Montpellier, Montpellier, France; UR22 AGIRs CIRAD, Campus International de Baillarguet, Montpellier, France; UMR CBGP (INRA/IRD/Cirad/Montpellier SupAgro), Campus International de Baillarguet, Montferrier-sur-Lez Cedex, France; and National Agricultural and Forestry Research Institute, Vientiane, Laos. [fvetspj@yahoo.com].

In this study, we investigated the molecular evidence of *Trypanosoma evansi* in wild rodents from Cambodia, Lao PDR and Thailand. Between November 2007 and June 2009, 1 664 rodents were trapped at eight sites representative of various ecological habitats. Of those animals, 94 were tested by direct microscopic blood examination, 633 using the card agglutination test for trypanosomes (CATT/*T. evansi*) and 145 by the polymerase chain

reaction (PCR) with two sets of primers: TRYP1 (amplifying ITS1 of ribosomal DNA of all trypanosomes) and TBR (amplifying satellite genomic DNA of *Trypanozoon* parasites). Using TRYP1, based on the size of the PCR products, 15 samples from the three countries were positive for *Trypanosoma lewisi* (two were confirmed by sequencing), and three were positive for *Trypanozoon* (one was confirmed by sequencing and three by TBR primers); the specificity of the primers failed as rodent DNA was amplified in some cases. Using TBR, six samples were positive for *Trypanozoon* (one was confirmed by sequencing); as *T. evansi* is the only species of the *Trypanozoon* sub-genus possibly present in Asian rodents, these results confirmed its presence in rodents from Thailand (*Rattus tanezumi*) and Cambodia (*R. tanezumi, Niviventer fulvescens* & *Maxomys surifer*). Further investigations are necessary to establish the situation in Lao PDR. None of the 16 samples most strongly positive to the CATT proved to be positive for *Trypanozoon* by PCR. The merits of the CATT for such studies were not confirmed. Studying the urban and rural circulation of these parasites in rodents will enable an evaluation of human exposure and infection risk, as human infections by *T. evansi* were recently described in India and by *T. lewisi* in India and Thailand. As sequencing PCR products is expensive, the development of new molecular and serological tools for rodents would be very useful.

16125. **Van Nieuwenhove, L., Buscher, P., Balharbi, F., Humbert, M., Dieltjens, T., Guisez, Y. & Lejon, V., 2012.** Identification of mimotopes with diagnostic potential for *Trypanosoma brucei gambiense v*ariant surface glycoproteins using human antibody fractions. *PLoS Neglected Tropical Diseases*, **6** (6): e1682.

Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; Dana-Farber Cancer Institute, Boston, Massachusetts, USA; Harvard Medical School, Boston, Massachusetts, USA; and Department of Biology, University of Antwerp, Antwerp, Belgium. [pbuscher@itg.be].

At present, screening of the population at risk for *Gambiense* human African trypanosomiasis (HAT) is based on detection of antibodies against native variant surface glycoproteins (VSGs) of *Trypanosoma brucei* (*T. b.) gambiense*. Drawbacks of these native VSGs include culture of infective *T. b. gambiense* trypanosomes in laboratory rodents necessary for production, and the exposure of non-specific epitopes that may cause cross-reactions. We therefore aimed at identifying peptides that mimic epitopes, hence called "mimotopes," and specific to *T. b. gambiense* VSGs and that may replace the native proteins in antibody detection tests. A Ph.D.-12™ peptide phage display library was screened with polyclonal antibodies from patient sera, previously affinity purified on VSG LiTat 1.3 or LiTat 1.5. The peptide sequences were derived from the DNA sequence of the selected phages and synthesised as biotinylated peptides. Respectively, eighteen and twenty different mimotopes were identified for VSG LiTat 1.3 and LiTat 1.5, of which six and five were retained for assessment of their diagnostic performance. Based on alignment of the peptide sequences on the original protein sequence of VSG LiTat 1.3 and 1.5, three additional peptides were synthesised. We evaluated the diagnostic performance of the synthetic peptides in indirect ELISA with 102 sera from HAT patients and 102 endemic negative controls. All mimotopes had areas under the curve (AUCs) of >-0.85, indicating their diagnostic potential. One peptide corresponding to the VSG LiTat 1.3 protein sequence also had an AUC of >-0.85, while the peptide based on the sequence of VSG LiTat 1.5 had an AUC of only 0.79. We delivered the proof of principle that mimotopes for *T. b. gambiense* VSGs with diagnostic potential can be selected by phage display using polyclonal human antibodies.

16126. **Van Nieuwenhove, L., Roge, S., Lejon, V., Guisez, Y. & Buscher, P., 2012.** Characterization of *Trypanosoma brucei gambiense* variant surface glycoprotein LiTat 1.5. *Genetics & Molecular Research*, **11** (2): 1260-1265.

Department of Biomedical Sciences, Institute of Tropical Medicine, Antwerp, Belgium; and Department of Biology, University of Antwerp, Antwerp, Belgium. [pbuscher@itg.be].

At present, all available diagnostic antibody detection tests for *Trypanosoma brucei gambiense* human African trypanosomiasis are based on predominant variant surface glycoproteins (VSGs), such as VSG LiTat 1.5. During investigations aiming at replacement of the native VSGs by recombinant proteins or synthetic peptides, the sequence of VSG LiTat 1.5 was derived from cDNA and direct N-terminal amino acid sequencing. Characterization of the VSG based on cysteine distribution in the amino acid sequence revealed an unusual cysteine pattern identical to that of VSG Kinu 1 of *T. b. brucei*. Even though both VSGs lack the third of four conserved cysteines typical for type A N-terminal domains, they can be classified as type A.

(b) PATHOLOGY AND IMMUNOLOGY

[See also **35**: 16046, 16048, 15050, 16058]

16127. **Amin, D. N., Vodnala, S. K., Masocha, W., Sun, B., Kristensson, K. & Rottenberg, M. E., 2012.** Distinct toll-like receptor signals regulate cerebral parasite load and interferon alpha/beta and tumour necrosis factor alpha-dependent T-cell infiltration in the brains of *Trypanosoma brucei*-infected mice. *Journal of Infectious Diseases*, **205** (2): 320-332.

Department of Neuroscience, Karolinska Institutet, Stockholm, Sweden; and Department of Applied Therapeutics, Faculty of Pharmacy, Kuwait University, Kuwait City [martin.rottenberg@ki.se].

The penetration of T cells and trypanosomes into the brain parenchyma is a major pathogenetic event in African trypanosomiasis. The role of innate immune responses in the penetration of T cells and *Trypanosoma brucei brucei* into the brain was studied in knockout mice by using double immunofluorescent staining and real-time polymerase chain reaction. We demonstrate that toll-like receptor (TLR)-MyD88-mediated signalling is required for T-cell and parasite penetration into the brain and microglial activation, besides controlling parasitemia and antigen-specific T-cell activation. Among different TLR-deficient mice studied, TLR9 mediated parasitaemia control and T-cell penetration into the brain. TLR-MyD88 signals increased levels of interferon (IFN) beta and tumour necrosis factor (TNF) alpha transcripts in the brains of infected mice and both TNF-alpha and IFN-alpha/beta receptors promoted T-cell and *Trypanosoma* infiltration into the brain parenchyma. Both resident and infiltrating inflammatory cells in the brain controlled parasite densities in a TLR2- and TLR9-MyD88-mediated manner. However, neither IFN-alpha/beta nor TNF-alpha contributed to parasite control in the brain. Our data indicate that innate immune TLR signals stimulate the expression of TNF-alpha and IFN-alpha/beta that initiate brain invasion of T cells and trypanosomes, and control *T. brucei brucei* load in the brain by molecules distinct from these.

16128. **Bruges, G., Betancourt, M., March, M., Sanchez, E. & Mijares, A., 2012.** Apoptotic-like activity of staurosporine in axenic cultures of *Trypanosoma evansi*. *Revista do Instituto de Medicina Tropical Sao Paulo*, **54** (2): 103-108.

Laboratorio de Fisiologia de Parasitos, Centro de Biofisica y Bioquimica, Instituto Venezolano de Investigaciones Cientificas, Caracas, Venezuela.

Trypanosoma evansi is a blood protozoan parasite of the genus *Trypanosoma* which is responsible for surra (trypanosomosis) in domestic and wild animals. This study addressed apoptotic-like features in *Trypanosoma evansi in vitro*. The mechanism of parasite death was investigated using staurosporine as an inducing agent. We evaluated its effects through several cytoplasmic features of apoptosis, including cell shrinkage, phosphatidylserine exposure, maintenance of plasma membrane integrity, and mitochondrial trans-membrane potential. For access to these features we have used flow cytometry and fluorescence microscopy with cultures in the stationary phase and adjusted to a density of 10^6 cells/mL. The apoptotic effect of staurosporine in *T. evansi* was evaluated at a final concentration of 20 nM. There was an increase of phosphatidylserine exposure, whereas mitochondrial potential was decreased. Moreover, no evidence was observed in this study of cell permeability increasing with staurosporine, suggesting the absence of a necrotic process. Additional studies are needed to elucidate the possible pathways associated with this form of cell death in this haemoparasite.

16129. **Bullard, W., Kieft, R., Capewell, P., Veitch, N. J., Macleod, A. & Hajduk, S. L., 2012.** Haptoglobin-haemoglobin receptor independent killing of African trypanosomes by human serum and trypanosome lytic factors. *Virulence*, **3** (1): 72-76.

Department of Biochemistry and Molecular Biology, University of Georgia, Athens, GA, USA; and Faculty of Veterinary Medicine, Wellcome Centre for Molecular Parasitology, Glasgow, UK. [shajduk@bmb.uga.edu].

The haptoglobin-haemoglobin receptor (HpHbR) of African trypanosomes plays a critical role in human innate immunity against these parasites. Localized to the flagellar pocket of the veterinary pathogen *Trypanosoma brucei brucei* this receptor binds trypanosome lytic factor-1 (TLF-1), a subclass of human high-density lipoprotein (HDL) facilitating endocytosis, lysosomal trafficking and subsequent killing. Recently, we found that group 1 *Trypanosoma brucei gambiense* does not express a functional HpHbR. We now show that loss of the TbbHpHbR reduces the susceptibility of *T. b. brucei* to human serum and TLF-1 by 100- and 10 000-fold, respectively. The relatively high concentrations of human serum and TLF-1 needed to kill trypanosomes lacking the HpHbR indicate that high affinity TbbHpHbR binding enhances the cytotoxicity; however, in the absence of TbbHpHbR, other receptors or fluid phase endocytosis are sufficient to provide some level of susceptibility. Human serum contains a second innate immune factor, TLF-2 that has been suggested to kill trypanosomes independently of the TbbHpHbR. We found that *T. b. brucei* killing by TLF-2 was reduced in TbbHpHbR-deficient cells but to a lesser extent than TLF-1. This suggests that both TLF-1 and TLF-2 can be taken up via the TbbHpHbR but that alternative pathways exist for the uptake of these toxins. Together the findings reported here extend our previously published studies and suggest that group 1 *T. b. gambiense* has evolved multiple mechanisms to avoid killing by trypanolytic human serum factors.

16130. **Caljon, G., Caveliers, V., Lahoutte, T., Stijlemans, B., Ghassabeh, G. H., Van Den Abbeele, J., Smolders, I., De Baetselier, P., Michotte, Y., Muyldermans, S.,**

Magez, S. & Clinckers, R., 2012. Using microdialysis to analyse the passage of monovalent nanobodies through the blood-brain barrier. *British Journal of Pharmacology*, **165** (7): 2341-2353.

Department of Animal Health, Institute of Tropical Medicine Antwerp, Antwerp, Belgium; Unit of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium; Department of Molecular and Cellular Interactions, VIB, Brussels, Belgium; Department of Nuclear Medicine, UZ Brussel and In Vivo Cellular and Molecular Imaging, ICMI, Vrije Universiteit Brussel, Brussels, Belgium; and Department of Pharmaceutical Chemistry and Drug Analysis, Center for Neuroscience, Vrije Universiteit Brussel, Brussels, Belgium. [ralph.clinckers@vub.ac.be].

Nanobodies are promising antigen-binding moieties for molecular imaging and therapeutic purposes because of their favourable pharmacological and pharmacokinetic properties. However, the capability of monovalent nanobodies to reach targets in the CNS remains to be demonstrated. We have assessed the blood-brain barrier permeability of Nb_An33, a nanobody against the *Trypanosoma brucei brucei* variant-specific surface glycoprotein (VSG). This analysis was performed in healthy rats and in rats that were in the encephalitic stage of African trypanosomiasis using intracerebral microdialysis, single photon emission computed tomography (SPECT) or a combination of both methodologies. This enabled the quantification of unlabelled and ^{99m} Tc-labelled nanobodies using respectively, a sensitive VSG-based nanobody-detection ELISA, radioactivity measurement in collected microdialysates and SPECT image analysis. The combined read-out methodologies showed that Nb_An33 was detected in the brain of healthy rats following i.v. injection, and that inflammation-induced damage to the blood-brain barrier, as in the late encephalitic stage of trypanosomiasis, significantly increased the efficiency of passage of the nanobody through this barrier. Complementing SPECT analyses with intracerebral microdialysis improved analysis of brain disposition. There is clear value in assessing penetration of the blood-brain barrier by monovalent nanobodies in models of CNS inflammation. Our data also suggest that rapid clearance from blood might hamper efficient targeting of specific nanobodies to the CNS. It is concluded that nanobodies can enter the brain parenchyma from the systemic circulation, especially in pathological conditions where the blood-brain barrier integrity is compromised.

16131. **Da Silva, A. S., Duck, M. R., Fanfa Vda, R., Otto, M. A., Nunes, J. T., Tonin, A. A., Jaques, J. A., Paim, F. C., Duarte, M. M. & Monteiro, S. G., 2012.** Trypanocidal activity of human plasma on *Trypanosoma evansi* in mice. *Revista Brasiliera de Parasitologia Veterinaria*, **21** (1): 55-59.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria-UFSM, Predio 20, Sala 4232, Campus Universitario, Camobi, CEP 97105-900, Santa Maria, RS, Brazil. [aleksandro_ss@yahoo.com.br].

This study aimed to test an alternative protocol with human plasma to control *Trypanosoma evansi* infection in mice. Plasma from an apparently 27-year-old healthy male, blood type A+, was used in the study. A concentration of 100 mg.dL^{-1} apolipoprotein L1 (APOL1) was detected in the plasma. Forty mice were divided into four groups with 10 animals each. Group A comprised uninfected animals. Mice from groups B, C and D were inoculated with a *T. evansi* isolate. Group B was used as a positive control. At three days p.i., the mice were administered intraperitoneally with human plasma. A single dose of 0.2 mL

plasma was given to those in group C. The mice from group D were administered five doses of 0.2 mL plasma with a 24 hours interval between the doses. Group B showed high increasing parasitaemia that led to their death within five days p.i. Both treatments eliminated parasites from the blood and increased the longevity of animals. An efficacy of 50 percent (group C) and 80 percent (group D) of human plasma trypanocidal activity was found using PCR. This therapeutic success was likely achieved in the group D due to their higher levels of APOL1 compared with group C.

16132. **Da Silva, A. S., Fanfa, V. R., Otto, M. A., Gressler, L. T., Tavares, K. C., Lazzarotto, C. R., Tonin, A. A., Miletti, L. C., Duarte, M. M. & Monteiro, S. G., 2011.** Susceptibility of mice to *Trypanosoma evansi* treated with human plasma containing different concentrations of apolipoprotein L-1. *Korean Journal of Parasitology*, **49** (4): 427-430.

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

The aim of this study was to test the susceptibility of mice to *Trypanosoma evansi* treated with human plasma containing different concentrations of apolipoprotein L-1 (APOL1). For this experiment, a strain of *T. evansi* and human plasma (plasmas 1, 2, and 3) from three clinically healthy adult males were used. The *in vivo* test used 50 mice divided in five groups (A to E) with 10 animals in each group. Animals of groups B to E were infected, and then treated with 0.2 ml of human plasma according to the following protocol: negative control (A); positive control (B), treatment with plasma 1 (C); treatment with plasma 2 (D); and treatment with plasma 3 (E). Mice treated with human plasma showed an increased longevity of 40.9 +/- 0.3 (C), 20 +/- 9.0 (D) and 35.6 +/- 9.3 (E) days compared with the control group (B) which was 4.3 +/- 0.5 days. The number of surviving mice and the number free of the parasite (blood smear and PCR negative) at the end of the experiment was 90 percent, 0 percent, and 60 percent for groups C, D, and E, respectively. The quantification of APOL1 was performed due to the large variation with the different source plasma. In plasmas 1, 2, and 3 concentrations of 194, 99, and 115 mg/dl of APOL1, respectively were detected. However, we believe that this difference in the treatment efficiency is related to the level of APOL1 in plasmas.

16133. **Da Silva, A. S., Oliveira, C. B., Bertoncheli, C. M., Santos, R. P., Beckmann, D. V., Wolkmer, P., Gressler, L. T., Tonin, A. A., Graca, D. L., Mazzanti, A., Lopes, S. T. & Monteiro, S. G., 2012.** Clinical signs and histopathology of brain, spinal cord and muscle of the pelvic limb of rats experimentally infected with *Trypanosoma evansi*. *Pathology- Research & Practice*, **208** (1): 39-44.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Brazil; and Department of Small Animals, Universidade Federal de Santa Maria, Brazil [aleksandro ss@yahoo.com.br].

The aim of this study was to investigate whether rats infected with *Trypanosoma evansi* had neurological and locomotor signs, as well as histological lesions in the central nervous system (CNS) and pelvic muscles. To carry out this study, 52 rats were used and divided into two groups. The animals in Group A (n=40) were infected with *T. evansi*, and the rats in Group B (n=12) were used as negative controls (non-infected). Neurological examination was performed at days 5, 15, 30 and 150 p.i. with eventual euthanasia of the rats. Samples of brain, spinal cord and skeletal muscle (biceps femoris and gastrocnemius muscles) were

collected. The neurological tests evaluated motor capacity, balance and pain sensitivity. At day 5 p.i. in subgroup A1, the rats showed high parasitaemia, became apathetic and presented with slow movements and signs of disorientation. After day 15 p.i. in subgroup A2 and day 30 p.i. in subgroup A3, no more clinical abnormalities were observed. Histologically, there was no damage to the CNS in these three subgroups, but within subgroup A3, mononuclear infiltration of the muscle was observed. Rats chronically infected (subgroup A4 - day 150 p.i.) showed muscle atrophy, walking dysfunction and paralysis of the hind limbs. Mild mononuclear inflammatory infiltrates and perivascular cuffs were observed in the CNS of some of the animals in subgroup A4. In these rats, severe muscle damage was observed in the skeletal muscle which included atrophy and loss of muscle fibres, multinucleated giant muscle cells, mononuclear myositis, Wallerian degeneration of the innervating fibres and mononuclear inflammatory infiltrate in the perineurium and adipose tissue. Based upon these findings, we conclude that infection by *T. evansi* in rats leads to muscle damage, which is probably the cause of the paralysis of hind limbs.

16134. **Da Silva, A. S., Oliveira, C. B., Rosa, L. D., Leal, C. A., Da Cruz, R. C., Thome, G. R., Athayde, M. L., Schetinger, M. R., Monteiro, S. G. & Lopes, S. T., 2012.** Influence of *Trypanosoma evansi* in adenine nucleotides and nucleoside concentration in serum and cerebral cortex of infected rats. *Experimental Parasitology*, **131** (1): 80-84.

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

This study aimed to evaluate the adenine nucleotides and nucleoside concentration in serum and cerebral cortex of rats infected with *Trypanosma evansi*. Each rat was intraperitoneally infected with 1 x 10^6 trypomastigotes suspended in cryopreserved blood (Group A; n = 18). Twelve animals were used as controls (Group B). The infected animals were monitored daily by blood smears. At days 4 and 20 p.i. serum and cerebral cortex were collected to measure the levels of ATP, ADP, AMP and adenosine by high performance liquid chromatography (HPLC). In serum there was a significant ($p < 0.05$) increase in the ATP, AMP and adenosine concentrations at days four and 20 p.i. in infected rats when compared with not-infected animals. Furthermore, in the cerebral cortex, a significant ($p < 0.05$) increase in the concentrations of ATP, AMP and a decrease adenosine levels at day four p.i. were recorded. At day 20 p.i. only increases in the AMP and adenosine concentrations in the cerebral cortex of infected rats were recorded when compared with non-infected animals. No differences in ADP concentration in serum and brain at days four and 20 p.i. were recorded and no change was observed histologically in the cerebral cortex of infected animals. The results allow us to conclude that infection with *T. evansi* in rats causes an increase in the concentrations of ATP, AMP and adenosine in serum and cerebral cortex at time periods evaluated. These alterations occurred as a result of *T. evansi* infection which involves neurotransmission, neuromodulation and immune response impairment and confirm the importance of the purinergic system in this pathology.

16135. **Ezeokonkwo, R. C., Ezeh, I. O., Onunkwo, J. I., Onyenwe, I. W., Iheagwam, C. N. & Agu, W. E., 2012.** Comparative serum biochemical changes in mongrel dogs following single and mixed infections of *Trypanosoma congolense* and *Trypanosoma brucei brucei. Veterinary Parasitology*. **Available online 23 May.**

Department of Veterinary Parasitology and Entomology, University of Nigeria, Nsukka, Nigeria; Department of Veterinary Public Health and Preventive Medicine,

University of Nigeria, Nsukka, Nigeria. [romanusezeokonkwo2002@yahoo.com].

The serum activities of alkaline phosphatase (AP), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and the serum levels of conjugated bilirubin (CB), blood urea nitrogen (BUN) and creatinine were studied following single and mixed infections of mongrel dogs with *Trypanosoma congolense* and *Trypanosoma brucei brucei*. Twenty mongrel dogs of both sexes aged between 3 and 6 months, and weighing between 2.5 and 5.9 kg were used for the study. The dogs were kept in clean metal cages in a fly-proof house and were fed and given water *ad libitum*. The twenty dogs were divided into four groups of five dogs each. Group I dogs were uninfected control, group II were infected with *T. congolense*, group III were infected with *T. brucei brucei* and group IV were infected with both *T. congolense* and *T. brucei brucei*. Each dog in the infected groups II and III was inoculated intraperitoneally (i/p) with 1.0 ml of PBS diluted blood containing $1.0x10^6$ trypanosomes whereas each infected dog in group IV (mixed infection) was inoculated with 0.5 ml of the PBS diluted blood containing $0.5x10^6$ *T. congolense* and 0.5 ml of the PBS diluted blood containing $0.5x10^6$ *T. brucei brucei* i/p. Parasites were detectable in the blood of the infected dogs in groups II, III, and IV 10-13 days p.i. with mean pre-patent periods (PP) of 12, 10, and 11 days respectively. Trypanosome infection caused significant ($P<0.05$) increases in the serum activities of AP, ALT, AST and in the serum levels of creatinine, CB, and BUN. The significant increases in the serum levels of CB, BUN, and creatinine and serum activities of AP and AST became noticeable from day seven p.i.in all the infected groups whereas that of ALT became noticeable from day 14 p.i. and increased continuously until the experiment was terminated. These increases, however, did not differ significantly ($p>0.05$) between the infected groups in most cases. It was thus concluded that single or mixed infection of mongrel dogs with *T. congolense* and *T. brucei brucei* resulted in significant increases in the serum activities of AP, AST, ALT and serum levels of creatinine, CB and BUN which in most cases did not differ significantly ($p>0.05$) among the infected groups.

16136. **Jackson, A. P., Berry, A., Aslett, M., Allison, H. C., Burton, P., Vavrova-Anderson, J., Brown, R., Browne, H., Corton, N., Hauser, H., Gamble, J., Gilderthorp, R., Marcello, L., McQuillan, J., Otto, T. D., Quail, M. A., Sanders, M. J., van Tonder, A., Ginger, M. L., Field, M. C., Barry, J. D., Hertz-Fowler, C. & Berriman, M., 2012.** Antigenic diversity is generated by distinct evolutionary mechanisms in African trypanosome species. *Proceedings of the National Academy of Sciences USA*, **109** (9): 3416-3421.

Wellcome Trust Sanger Institute, Hinxton, Cambridge CB10 1SA, UK; Department of Pathology, University of Cambridge, Cambridge CB2 1QP, UK; Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity, and Inflammation, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK; Division of Biomedical and Life Sciences, School of Health and Medicine, Lancaster University, Lancaster LA1 4YQ, UK; and Centre for Genomic Research, Institute of Integrative Biology, University of Liverpool, Liverpool L69 7ZB, UK. [andrew.jackson@sanger.ac.uk].

Antigenic variation enables pathogens to avoid the host immune response by continual switching of surface proteins. The protozoan blood parasite *Trypanosoma brucei* causes human African trypanosomiasis ("sleeping sickness") across sub-Saharan Africa and is a model system for antigenic variation, surviving by periodically replacing a monolayer of variant surface glycoproteins (VSG) that covers its cell surface. We compared the genome of *Trypanosoma brucei* with two closely related parasites *Trypanosoma congolense* and

Trypanosoma vivax, to reveal how the variant antigen repertoire has evolved and how it might affect contemporary antigenic diversity. We reconstruct VSG diversification showing that *Trypanosoma congolense* uses variant antigens derived from multiple ancestral VSG lineages, whereas in *Trypanosoma brucei* VSG have recent origins, and ancestral gene lineages have been repeatedly co-opted to novel functions. These historical differences are reflected in fundamental differences between species in the scale and mechanism of recombination. Using phylogenetic incompatibility as a metric for genetic exchange, we show that the frequency of recombination is comparable between *Trypanosoma congolense* and *Trypanosoma brucei* but is much lower in *Trypanosoma vivax*. Furthermore, in showing that the C-terminal domain of *Trypanosoma brucei* VSG plays a crucial role in facilitating exchange, we reveal substantial species differences in the mechanism of VSG diversification. Our results demonstrate how past VSG evolution indirectly determines the ability of contemporary parasites to generate novel variant antigens through recombination and suggest that the current model for antigenic variation in *Trypanosoma brucei* is only one means by which these parasites maintain chronic infections.

16137. **Jia, Y., Guo, L., Zhao, X. & Suo, X., 2012.** VSG 117 gene is conservatively present and early expressed in *Trypanosma evansi* YNB stock. *Experimental Parasitology*, **131** (1): 75-79.

National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing 100193, China; and National Animal Protozoa Laboratory & College of Veterinary Medicine, China Agricultural University, Beijing 100193, China. [jarregon@163.com].

African trypanosomes, including *Trypanosoma brucei* and the closely related species *Trypanosoma evansi*, are flagellated unicellular parasites that proliferate extracellularly in the mammalian bloodstream and tissue spaces. They evade host immune system by periodically switching their variant surface glycoprotein (VSG) coat. Each trypanosome possesses a vast archive of VSGs with distinct sequence identity and different strains contain different archive of VSGs. VSG 117 was reported as a widespread VSG detected in the genomes of all *T. brucei* strains. In this study, the presence and expression of VSG 117 gene were observed in *T. evansi* YNB stock by RT-PCR with VSG-specific primers. We further confirmed that this VSG tends to be expressed in the early stage of *T. evansi* infections (on day 12-15) by immuno-screening the previously isolated infected blood samples. It is possible that the VSG 117 gene evolved and spread through the African trypanosome population via genetic exchange, before *T. evansi* lost its ability to infect the tsetse fly. Our findings provide evidence of the close evolutionary relationship between *T. evansi* and *T. brucei* in the terms of VSG genes.

16138. **Kangethe, R. T., Boulange, A. F., Coustou, V., Baltz, T. & Coetzer, T. H., 2012.** *Trypanosoma brucei brucei* oligopeptidase B null mutants display increased prolyl oligopeptidase-like activity. *Molecular & Biochemical Parasitology*, **182** (1-2): 7-16.

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

African trypanosomosis is a parasitic disease in man and animals caused by protozoan parasites of the genus *Trypanosoma*. Nagana, the cattle form of the disease, is caused by *Trypanosoma congolense*, *Trypanosoma vivax* and *Trypanosoma brucei brucei*. An option for developing vaccines and chemotherapeutic agents against trypanosomosis is to target

pathogenic factors released by the parasite during infection, namely an "anti-disease" approach. One such pathogenic factor is oligopeptidase B (TbOPB), a trypanosome peptidase that hydrolyses Arg/Lys containing peptides smaller than 30 amino acid residues and is suspected to be involved in the hormonal deregulation associated with the disease. To better understand the role TbOPB plays in parasite physiology and host pathogenesis, oligopeptidase B null mutant parasites (Deltaopb) were generated in the *T. b. brucei* Lister 427 strain. Deltaopb *Trypanosoma brucei* parasites grew at a significantly faster rate *in vitro*, and were as virulent as wild type strains during infection in mice. Immunohistopathology of infected mouse testes revealed Deltaopb parasites in extra vascular regions showing that TbOPB is not involved in assisting *T. brucei* parasites to cross microvascular endothelial cells. Gelatine gel analysis of Deltaopb null mutants showed an increase in discrete cysteine peptidase activities when compared with wild type strains. Enzymatic activity assays were carried out to identify how closely related oligopeptidases are affected by TbOPB gene deletion. A significant increase of *T. brucei* prolyl oligopeptidase (TbPOP) activity was observed, but no concomitant increase in TbPOP protein levels, suggesting that a POP-like enzyme might compensate for a loss in OPB activity in Deltaopb null mutants.

16139. **MacGregor, P., Szoor, B., Savill, N. J. & Matthews, K. R., 2012.** Trypanosomal immune evasion, chronicity and transmission: an elegant balancing act. *Nature Reviews Microbiology*, **10** (6): 431-438.

Centre for Immunity, Infection and Evolution, Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh EH9 3JT, UK. [keith.matthews@ed.ac.uk].

During their life cycle, trypanosomes must overcome conflicting demands to ensure their survival and transmission. First, they must evade immunity without overwhelming the host. Second, they must generate and maintain transmission stages at sufficient levels to allow passage into their tsetse vector. Finally, they must rapidly commit to onward development when they enter the tsetse fly. On the basis of recent quantification and modelling of *Trypanosoma brucei* infection dynamics, we propose that the interplay between immune evasion and development achieves both infection chronicity and transmissibility. Moreover, we suggest that a novel form of bistable regulation ensures developmental commitment on entry into the tsetse fly midgut.

16140. **Mekata, H., Konnai, S., Mingala, C. N., Abes, N. S., Gutierrez, C. A., Dargantes, A. P., Witola, W. H., Inoue, N., Onuma, M., Murata, S. & Ohashi, K., 2012.** Kinetics of regulatory dendritic cells in inflammatory responses during *Trypanosoma evansi* infection. *Parasite Immunology*, **34** (6): 318-329.

Department of Disease Control, Graduate School of Veterinary Medicine, Hokkaido University, Sapporo, Japan; Animal Health Laboratory, Philippine Carabao Center, Science City of Munoz, Nueva Ecija, Philippines; College of Veterinary Medicine, Central Mindanao University, Bukidnon, Philippines; Department of Agricultural and Environmental Sciences, Tuskegee University, Tuskegee, AL, USA; and National Research Centre for Protozoan Diseases, Obihiro University of Agriculture and Veterinary Medicine, Obihiro, Japan. [okazu@vetmed.hokudai.ac.jp].

Trypanosoma evansi (*T. evansi*) causes a wasting disease in almost all mammals. *Trypanosoma evansi* infection gives rise to the inflammatory responses that contribute to the development of inflammation-associated tissue injury. To determine what kinds of

inflammatory molecules play roles in the pathogenicity of *T. evansi* infection, polymerase chain reaction array analysis was performed on samples from infected and uninfected mice. The inflammatory cytokine and chemokine storm, caused mainly by macrophages, was observed. On the other hand, the expression levels of Ccl8 and Il10 in splenocytes were also markedly increased. These results suggested an augmentation in the number and activity of regulatory dendritic cells (DCs). Therefore, the kinetics of regulatory DCs in *T. evansi*-infected mice were investigated. During *T. evansi* infection, the regulatory DCs became prevalent, reducing the amount of inflammatory DCs. Interestingly, when the regulatory DCs were implanted into *T. evansi*-infected mice, the survival was prolonged, and the expression levels of inflammatory molecules were suppressed. Taken together, these results showed that a subset of regulatory DCs acted as a potential regulator of the inflammatory responses.

16141. **Oliveira, C. B., da Silva, A. S., Souza, V. C., Costa, M. M., Jaques, J. A., Leal, D. B., Lopes, S. T. & Monteiro, S. G., 2012.** NTPDase activity in lymphocytes of rats infected by *Trypanosoma evansi*. *Parasitology*, **139** (2): 232-236.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Brazil; Department of Small Animals, Universidade Federal de Santa Maria, Brazil; and Department of Chemistry, Universidade Federal de Santa Maria, Brazil. [camilabelmontevet@yahoo.com.br].

Trypanosoma evansi is the aetiological agent of trypanosomosis in domestic animals. In this pathology, an inflammatory response can be observed and, as a consequence, an increase of extracellular adenine nucleotides such as ATP. These nucleotide concentrations are regulated by ectoenzymes such as NTPDase (EC 3.6.1.5, CD39), which catalyses the hydrolysis of ATP and ADP into AMP. In this study, the activity of NTPDase in lymphocytes of rats experimentally infected with *T. evansi* was evaluated. The animals were inoculated with the parasite and monitored by blood smear on a daily basis. The animals were then were divided into four groups according to the degree of parasitaemia and period of infection. The blood collections for enzyme analysis and lymphocyte count were performed on the 3rd (beginning of infection), 5th (acute infection) and 15th (chronic infection) days p.i. The control group was composed of non-infected animals. In the infected group a decrease in ATP hydrolysis (36 percent) was observed on day three p.i. and a decrease in ADP hydrolysis (62 percent) was observed on day five p.i. when compared with the control. On the 15th day p.i., increases in ATP (94 percent) and ADP (50 percent) hydrolysis were observed in the infected group. Considering these data it is suggested that NTPDase activity is altered on the surface of lymphocytes of rats infected with *T. evansi* at different time points of infection.

16142. **Salmon, D., Bachmaier, S., Krumbholz, C., Kador, M., Gossmann, J. A., Uzureau, P., Pays, E. & Boshart, M., 2012.** Cytokinesis of *Trypanosoma brucei* bloodstream forms depends on expression of adenylyl cyclases of the ESAG4 or ESAG4-like subfamily. *Molecular Microbiology*, **84** (2): 225-242.

Laboratory of Molecular Parasitology, Institute of Molecular Biology and Medicine, Université Libre de Bruxelles, 12, rue des Professeurs Jeener et Brachet, B-6041 Gosselies, Belgium. [salmon@bioqmed.ufrj.br].

Antigenic variation of the parasite *Trypanosoma brucei* operates by monoallelic expression of a variant surface glycoprotein (VSG) from a collection of multiple telomeric expression sites (ESs). Each of these ESs harbours a long polycistronic transcription unit containing several expression site-associated genes (ESAGs). ESAG4 copies encode bloodstream stage-

specific adenylyl cyclases (AC) and belong to a larger gene family of around 80 members, the majority of which, termed genes related to ESAG4 (GRESAG4s), are not encoded in ESs and are expressed constitutively in the life cycle. Here we report that ablation of ESAG4 from the active ES did not affect parasite growth, neither in culture nor upon rodent infection, and did not significantly change total AC activity. In contrast, inducible RNAi-mediated knock-down of an AC subfamily that includes ESAG4 and two ESAG4-like GRESAG4 (ESAG4L) genes, decreased total AC activity and induced a lethal phenotype linked to impaired cytokinesis. In the Delta esag4 line compensatory upregulation of apparently functionally redundant ESAG4L genes was observed, suggesting that the ESAG4/ESAG4L-subfamily ACs are involved in the control of cell division. How deregulated adenylyl cyclases or cAMP might impair cytokinesis is discussed.

16143. **Savage, A. F., Cerqueira, G. C., Regmi, S., Wu, Y., El Sayed, N. M. & Aksoy, S., 2012.** Transcript expression analysis of putative *Trypanosoma brucei* GPI-anchored surface proteins during development in the tsetse and mammalian hosts. *PLoS Neglected Tropical Diseases*, **6** (6): e1708.

Division of Epidemiology of Microbial Diseases, Yale School of Public Health, Yale University, New Haven, Connecticut, USA; Department of Cell Biology and Molecular Genetics, Maryland Pathogen Research Institute (MPRI), University of Maryland, College Park, Maryland, USA; and Center for Bioinformatics and Computational Biology, College of Chemical & Life Sciences, University of Maryland, College Park, Maryland, USA. [serap.aksoy@yale.edu].

Human African trypanosomiasis is a devastating disease caused by the parasite *Trypanosoma brucei*. Trypanosomes live extracellularly in both the tsetse fly and the mammal. Trypanosome surface proteins can directly interact with the host environment, allowing parasites to effectively establish and maintain infections. Glycosylphosphatidylinositol (GPI) anchoring is a common posttranslational modification associated with eukaryotic surface proteins. In *T. brucei*, three GPI-anchored major surface proteins have been identified: variant surface glycoproteins (VSGs), procyclic acidic repetitive protein (PARP or procyclins), and *brucei* alanine rich proteins (BARP). The objective of this study was to select genes encoding predicted GPI-anchored proteins with unknown function(s) from the *T. brucei* genome and characterize the expression profile of a subset during cyclical development in the tsetse and mammalian hosts. An initial *in silico* screen of putative *T. brucei* proteins by Big PI algorithm identified 163 predicted GPI-anchored proteins, 106 of which had no known functions. Application of a second GPI-anchor prediction algorithm (FragAnchor), signal peptide and trans-membrane domain prediction software resulted in the identification of 25 putative hypothetical proteins. Eighty-one gene products with hypothetical functions were analysed for stage-regulated expression using semi-quantitative RT-PCR. The expression of most of these genes was found to be upregulated in trypanosomes infecting tsetse salivary gland and proventriculus tissues, and 38 percent were specifically expressed only by parasites infecting salivary gland tissues. Transcripts for all of the genes specifically expressed in salivary glands were also detected in mammalian infective metacyclic trypomastigotes, suggesting a possible role for these putative proteins in invasion and/or establishment processes in the mammalian host. These results represent the first large-scale report of the differential expression of unknown genes encoding predicted *T. brucei* surface proteins during the complete developmental cycle. This knowledge may form the foundation for the development of future novel transmission blocking strategies against metacyclic parasites.

16144. **Seke Etet, P. F., Palomba, M., Colavito, V., Grassi-Zucconi, G., Bentivoglio, M. & Bertini, G., 2012.** Sleep and rhythm changes at the time of *Trypanosoma brucei* invasion of the brain parenchyma in the rat. *Chronobiology International*, **29** (4): 469-481.

Department of Neurological Sciences, University of Verona, Italy. [paul.seke@gmail.com].

Human African trypanosomiasis (HAT), or sleeping sickness, is a severe disease caused by *Trypanosoma brucei* (*T. b.*). The disease hallmark is sleep alterations. Brain involvement in HAT is a crucial pathogenetic step for disease diagnosis and therapy. In this study, a rat model of African trypanosomiasis was used to assess changes of sleep-wake, rest-activity, and body temperature rhythms in the time window previously shown as crucial for brain parenchyma invasion by *T. b.* to determine potential biomarkers of this event. Chronic radiotelemetric monitoring in Sprague-Dawley rats was used to continuously record electroencephalogram, electromyogram, rest-activity, and body temperature in the same animals before (baseline recording) and after infection. Rats were infected with *T. b. brucei*. Data were acquired from 1 to 20 days after infection (parasite neuroinvasion initiates at 11-13 days p.i. in this model), and were compared with baseline values. Sleep parameters were manually scored from electroencephalographic-electromyographic tracings. Circadian rhythms of sleep time, slow-wave activity, rest-activity, and body temperature were studied using cosinor rhythmometry. Results revealed alterations of most of the analysed parameters. In particular, sleep pattern and sleep-wake organization plus rest-activity and body temperature rhythms exhibited early quantitative and qualitative alterations, which became marked around the time interval crucial for parasite neuroinvasion or shortly after. Data derived from actigrams showed close correspondence with those from hypnograms, suggesting that rest-activity could be useful to monitor sleep-wake alterations in African trypanosomiasis.

16145. **Sengupta, P. P., Balumahendiran, M., Balamurugan, V., Rudramurthy, G. R. & Prabhudas, K., 2012.** Expressed truncated N-terminal variable surface glycoprotein (VSG) of *Trypanosoma evansi* in *E. coli* exhibits immuno-reactivity. *Veterinary Parasitology*, **187** (1-2): 1-8.

Project Directorate on Animal Disease Monitoring and Surveillance (PD_ADMAS), Hebbal, Bengaluru, Karnataka 560024, India. [pinakiprasad s@rediffmail.com].

The variant surface glycoprotein (VSG) of trypanosome is an important part of its body surface coat, which is expressed in early, middle and late stages of infection contributing a major diagnostic value. In the present study, the 5' end of the partial VSG gene sequences (681 bp) encoding N-terminal protein of RoTat 1.2 VSG (227 amino acid) was amplified, cloned into pET32a vector, and expressed in prokaryotic system. The fused His-tagged expressed VSG protein (43 kDa) of *Trypanosoma evansi* was characterized by SDS-PAGE and immunoblotting using hyperimmune/immune sera raised against buffalo, dog, lion and leopard isolates of *T. evansi*. The expressed protein remained immunoreactive with all the sera combinations. The animals immunized with whole cell lysate or recombinant protein showed similar antibody reactions in ELISA and CATT (card agglutination test for trypanosomiasis). This study suggests that the expressed recombinant truncated VSG is important for a possible use in serodiagnosis of surra.

16146. **Van den Bossche, J., Laoui, D., Morias, Y., Movahedi, K., Raes, G., De Baetselier, P. & Van Ginderachter, J. A., 2012.** Claudin-1, claudin-2 and claudin-11 genes differentially associate with distinct types of anti-inflammatory macrophages *in vitro* and with parasite- and tumour-elicited macrophages *in vivo*. *Scandinavian Journal of Immunology*, **75** (6): 588-598.

Myeloid Cell Immunology Laboratory, VIB, Brussels, Belgium; and Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium. [jo.vanginderachter@vib-vub.be].

Macrophages altered by various Th2-associated and anti-inflammatory mediators - including IL-4 and IL-13 [inducing alternatively activated macrophages (AAMs)], IL-10 and TGF-beta- were generically termed M2. However, markers that discriminate between AAMs and other M2 remain scarce. We previously described E-cadherin as a marker for AAMs, permitting these macrophages to fuse upon IL-4 stimulation. To identify novel potential contributors to macrophage fusion, we assessed the effect of IL-4 on other adherens and tight junction-associated components. We observed an induction of claudin-1 (Cldn1), Cldn2 and Cldn11 genes by IL-4 in different mouse macrophage populations. Extending our findings to other stimuli revealed Cldn1 as a mainly TGF-beta-induced gene and showed that Cldn11 is predominantly associated with IL-4-induced AAMs. Cldn2 is upregulated by diverse stimuli and is not associated with a specific macrophage activation state *in vitro*. Interestingly, different claudin genes preferentially associate with M2 from distinct diseases. While Cldn11 is predominantly expressed in AAMs from helminth-infected mice, Cldn1 is the major macrophage claudin during chronic trypanosomiasis and Cldn2 dominates in tumour-associated macrophages. Overall, we identified Cldn1, Cldn2 and Cldn11 as genes that discriminate between diverse types of M2.

16147. **Weirather, J. L., Wilson, M. E. & Donelson, J. E., 2012.** Mapping of VSG similarities in *Trypanosoma brucei*. *Molecular & Biochemical Parasitology*, **181** (2): 141-152.

Interdisciplinary Program in Genetics, University of Iowa, Iowa City, IA 52240, USA.

The protozoan parasite *Trypanosoma brucei* switches its variant surface glycoprotein (VSG) to subvert its mammalian hosts' immune responses. The *T. brucei* genome contains as many as 1 600 VSG genes (VSGs), but most are silent noncoding pseudogenes. Only one functional VSG, located in a telomere-linked expression site, is transcribed at a time. Silent VSGs are copied into a VSG expression site through gene conversion. Truncated gene conversion events can generate new mosaic VSGs with segments of sequence identity to other VSGs. To examine the VSG family sub-structure within which these events occur, we combined the available VSG sequences and annotations with scripted BLAST searches to map the relationships among VSGs in the *T. brucei* genome. Clusters of related VSGs were visualized in 2- and 3-dimensions for different N- and C-terminal regions. Five types of N-termini (N1-N5) were observed, within which gene recombinational events are likely to occur, often with fully-coding “functional” or “atypical” VSGs centrally located between more dissimilar VSGs. Members of types N1, N3 and N4 are most closely related in the middle of the N-terminal region, whereas type N2 members are more similar near the N-terminus. Some preference occurs in pairing between specific N- and C-terminal types. Statistical analyses indicated no overall tendency for more related VSGs to be located closer in the genome than less related VSGs, although exceptions were noted. Many potential mosaic gene formation events within each N-terminal type were identified, contrasted by only

one possible mosaic gene formation between N-terminal types (N1 and N2). These data suggest that mosaic gene formation is a major contributor to the overall VSG diversity, even though gene recombinational events between members of different N-terminal types occur only rarely.

(c) CHEMOTHERAPEUTICS

[See also 3**5**: 16038, 16041, 16043, 16045, 16049, 16051, 16054, 16055, 16056]

16148. **Abdelwahab, N. Z., Crossman, A. T., Sullivan, L., Ferguson, M. A. & Urbaniak, M. D., 2012.** Inhibitors incorporating zinc-binding groups target the GlcNAc-PI de-N-acetylase in *Trypanosoma brucei*, the causative agent of African sleeping sickness. *Chemical Biology & Drug Design*, **79** (3): 270-278.

Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee, UK. [m.d.urbaniak@dundee.ac.uk].

16149. **Almerico, A. M., Tutone, M., Guarcello, A. & Lauria, A., 2012.** *In vitro* and *in silico* studies of polycondensed diazine systems as anti-parasitic agents. *Bioorganic & Medicinal Chemistry Letters*, **22** (2): 1000-1004.

Dipartimento di Scienze e Tecnologie Molecolari e Biomolecolari (STEMBIO) Sezione di Chimica Farmaceutica e Biologica, Universita di Palermo, Via Archirafi, 32, 90123 Palermo, Italy. [annamaria.almerico@unipa.it].

Parasitic diseases caused by protozoan agents are more relevant today than ever. Recently, we synthesized several polycondensed diazine derivatives by means of 1,3-dipolar cycloaddition reactions. A broad selection of these compounds was submitted to *in vitro* biological screening against *Plasmodium falciparum, Leishmania infantum, Trypanosoma brucei*, and *Trypanosoma cruzi*, and were active at the µmolar level. Induced fit docking/MM-GBSA studies were performed giving interesting indications about the probable mechanism of action of the most active compounds.

16150. **Al-Musayeib, N. M. N., Mothana, R. A. R., Matheeussen, A. A., Cos, P. P. & Maes, L. L., 2012.** *In vitro* antiplasmodial, antileishmanial and antitrypanosomal activities of selected medicinal plants used in the traditional Arabian Peninsula region. *BMC Complementary & Alternative Medicine*, **12** (1): 49.

Department of Pharmacognosy, College of Pharmacy, King Saud University, P.O. Box 2457, Riyadh 11451, Saudi Arabia; Laboratory for Microbiology, Parasitology and Hygiene (LMPH), Faculty of Pharmaceutical, Biomedical and Veterinary Sciences, Antwerp University, Groenenborgerlaan 171, B-2020 Antwerp, Belgium. [r_mothana@yahoo.co].

Worldwide, particularly in developing countries, a large proportion of the population is at risk from tropical parasitic diseases. Several medicinal plants are still used traditionally against protozoal infections in Yemen and Saudi Arabia. Thus the present study investigated the *in vitro* antiprotozoal activity of twenty five plants collected from the Arabian Peninsula. Plant materials were extracted with methanol and screened *in vitro* against erythrocytic schizonts of *Plasmodium falciparum*, intracellular amastigotes of *Leishmania infantum* and *Trypanosoma cruzi* and free trypomastigotes of *T. brucei*. Cytotoxic activity was determined against MRC-5 cells to assess selectivity. The criteria for activity were an $IC_{50} < 10$ µg/ml (<5

µg/ml for *T. brucei*) and a selectivity index of >4. Antiplasmodial activity was found in the extracts of *Chrozophora oblongifolia, Ficus ingens, Lavandula dentata* and *Plectranthus barbatus*. Amastigotes of *T. cruzi* were affected by *Grewia erythraea, L. dentata, Tagetes minuta* and *Vernonia leopoldii*. Activity against *T. brucei* was obtained in *G. erythraea, L. dentata, P. barbatus* and *T. minuta*. No relevant activity was found against *L. infantum*. High levels of cytotoxicity (MRC-5 IC_{50} < 10 µg/ml) and hence non-specific activities were noted in *Cupressus empervirens, Kanahia laniflora* and *Kniphofia sumarae*. The results confirm that medicinal plants can be promising sources of natural products with antiprotozoal activity potential. The results support to some extent the traditional uses of some plants for the treatment of parasitic protozoal diseases.

16151. **Alsford, S., Eckert, S., Baker, N., Glover, L., Sanchez-Flores, A., Leung, K. F., Turner, D. J., Field, M. C., Berriman, M. & Horn, D., 2012.** High-throughput decoding of antitrypanosomal drug efficacy and resistance. *Nature*, **482** (7384): 232-236.

London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, UK; The Wellcome Trust Sanger Institute, Hinxton, Cambridge, CB10 1SA, UK; and Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QP, UK.

The concept of disease-specific chemotherapy was developed a century ago. Dyes and arsenical compounds that displayed selectivity against trypanosomes were central to this work, and the drugs that emerged remain in use for treating human African trypanosomiasis (HAT). The importance of understanding the mechanisms underlying selective drug action and resistance for the development of improved HAT therapies has been recognized, but these mechanisms have remained largely unknown. Here we use all five current HAT drugs for genome-scale RNA interference target sequencing (RIT-seq) screens in *Trypanosoma brucei*, revealing the transporters, organelles, enzymes and metabolic pathways that function to facilitate antitrypanosomal drug action. RIT-seq profiling identifies both known drug importers and the only known pro-drug activator, and links more than fifty additional genes to drug action. A bloodstream stage-specific invariant surface glycoprotein (ISG75) family mediates suramin uptake, and the AP1 adaptin complex, lysosomal proteases and major lysosomal transmembrane protein, as well as spermidine and N-acetylglucosamine biosynthesis, all contribute to suramin action. Further screens link ubiquinone availability to nitro-drug action, plasma membrane P-type H^+-ATPases to pentamidine action, and trypanothione and several putative kinases to melarsoprol action. We also demonstrate a major role for aquaglyceroporins in pentamidine and melarsoprol cross-resistance. These advances in our understanding of mechanisms of antitrypanosomal drug efficacy and resistance will aid the rational design of new therapies and help to combat drug resistance, and provide unprecedented molecular insight into the mode of action of antitrypanosomal drugs.

16152. **Aragao, E. A., Vieira, D. S., Chioato, L., Ferreira, T. L., Lourenzoni, M. R., Silva, S. R. & Ward, R. J., 2012.** Characterization of suramin binding sites on the human group IIA secreted phospholipase A2 by site-directed mutagenesis and molecular dynamics simulation. *Archives of Biochemistry & Biophysics*, **519** (1): 17-22.

Department of Chemistry, FFCLRP-USP, Universidade de Sao Paulo, Brazil; Department of Biochemistry and Immunology, FMRP-USP, Universidade de São

Paulo, Brazil; Verdartis – Desenvolvimento Biotecnológico Ltda-ME, SP, Brazil; and Computer Science Departament, Universidade Federal de São Carlos, SP, Brazil. [rjward@fmrp.usp.br].

Suramin is a polysulphonated naphthylurea with inhibitory activity against the human secreted group IIA phospholipase A2 (hsPLA2GIIA), and we have investigated suramin binding to recombinant hsPLA2GIIA using site-directed mutagenesis and molecular dynamics (MD) simulations. The changes in suramin binding affinity of 13 cationic residue mutants of the hsPLA2GIIA were strongly correlated with alterations in the inhibition of membrane damaging activity of the protein. Suramin binding to hsPLA2GIIA was also studied by MD simulations, which demonstrated that altered intermolecular potential energy of the suramin/mutant complexes was a reliable indicator of affinity change. Although residues in the C-terminal region play a major role in the stabilization of the hsPLA2GIIA/suramin complex, attractive and repulsive hydrophobic and electrostatic interactions with residues throughout the protein together with the adoption of a bent suramin conformation, all contribute to the stability of the complex. Analysis of the hsPLA2GIIA/suramin interactions allows the prediction of the properties of suramin analogues with improved binding and higher affinities which may be candidates for novel phospholipase A2 inhibitors.

16153. **Aran, V. J., Kaiser, M. & Dardonville, C., 2012.** Discovery of nitroheterocycles active against African trypanosomes. *In vitro* screening and preliminary SAR studies. *Bioorganic & Medicinal Chemistry Letters*, **22** (14): 4506-4516.

Instituto de Quimica Medica, CSIC, Juan de la Cierva 3, E-28006 Madrid, Spain; Swiss Tropical and Public Health Institute, Socinstrasse, 57, CH-4002 Basel, Switzerland; and University of Basel, Basel, Switzerland. [dardonville@iqm.csic.es].

A selection of 76 nitroheterocycles and related compounds from our in-house compound library was screened *in vitro* against the parasite *Trypanosoma brucei rhodesiense*, causative agent of human African trypanosomiasis (HAT). The unspecific cytotoxicity of the compounds was also evaluated against rat myoblast L6-cells to measure the selectivity of the compounds towards the parasite. This screening revealed some preliminary structure-activity relationships (SAR) among the series, and six hit compounds showing interesting activity (IC_{50}10 µM) and fair selectivity (SI>17). The 7-nitroquinoxalin-2-one and 5-nitroindazole scaffold derivatives 58 and 35, respectively, are particularly interesting because of their established oral bioavailability in mice. These hits represent interesting starting points for a medicinal project aimed at identifying the SAR behind this class of compounds.

16154. **Audisio, D., Messaoudi, S., Cojean, S., Peyrat, J. F., Brion, J. D., Bories, C., Huteau, F., Loiseau, P. M. & Alami, M., 2012.** Synthesis and antikinetoplastid activities of 3-substituted quinolinones derivatives. *European Journal of Medicinal Chemistry*, **52**: 44-50.

Université Paris-Sud, CNRS, BioCIS-UMR 8076, Laboratoire de Chimie Thérapeutique, LabEx LERMIT, Faculté de Pharmacie, 5 rue J.-B. Clement, Chatenay-Malabry, F-92296, France; and Université Paris-Sud, CNRS, BioCIS-UMR 8076, Chimiothérapie Antiparasitaire, LabEx LERMIT, Faculté de Pharmacie, 5 rue J.-B. Clément, Châtenay-Malabry, F-92296, France. [samir.messaoudi@u-psud.fr].

16155. **Brand, S., Cleghorn, L. A., McElroy, S. P., Robinson, D. A., Smith, V. C., Hallyburton, I., Harrison, J. R., Norcross, N. R., Spinks, D., Bayliss, T., Norval, S., Stojanovski, L., Torrie, L. S., Frearson, J. A., Brenk, R., Fairlamb, A. H., Ferguson, M. A., Read, K. D., Wyatt, P. G. & Gilbert, I. H., 2012.** Discovery of a novel class of orally active trypanocidal N-myristoyltransferase inhibitors. *Journal of Medicinal Chemistry*, **55** (1): 140-152.

Drug Discovery Unit, Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Sir James Black Centre, Dundee, DD1 5EH, UK. [i.h.gilbert@dundee.ac.uk].

N-myristoyltransferase (NMT) represents a promising drug target for human African trypanosomiasis (HAT), which is caused by the parasitic protozoan *Trypanosoma brucei*. We report the optimization of a high throughput screening hit (1) to give a lead molecule DDD85646 (63), which has potent activity against the enzyme (IC_{50} = 2 nM) and *T. brucei* (EC_{50} = 2 nM) in culture. The compound has good oral pharmacokinetics and cures rodent models of peripheral HAT infection. This compound provides an excellent tool for validation of *T. brucei* NMT as a drug target for HAT as well as a valuable lead for further optimization.

16156. **Calligari, P. A., Salgado, G. F., Pelupessy, P., Lopes, P., Ouazzani, J., Bodenhausen, G. & Abergel, D., 2012.** Insights into internal dynamics of 6-phosphogluconolactonase from *Trypanosoma brucei* studied by nuclear magnetic resonance and molecular dynamics. *Proteins*, **80** (4): 1196-1210.

Departement de Chimie, Ecole Normale Supérieure, Paris, France; Université Pierre-et-Marie Curie, Place Jussieu, 75005 Paris, France; UMR 7203 CNRS, 24 rue Lhomond, 75231 Paris Cedex 05, France; Institut de Chimie des Substances Naturelles, UPR 2301 CNRS, avenue de la Terrasse, 91198 Gif sur Yvette Cedex, France; and Institut de Sciences et Ingénierie Chimiques, Ecole Polytechnique Fédérale de Lausanne, Batochime, 1015 Lausanne, Switzerland. [g.salgado@iecb.u-bordeaux.fr].

16157. **Caminos, A. P., Panozzo-Zenere, E. A., Wilkinson, S. R., Tekwani, B. L. & Labadie, G. R., 2012.** Synthesis and antikinetoplastid activity of a series of N,N'-substituted diamines. *Bioorganic & Medicinal Chemistry Letters*, **22** (4): 1712-1715.

Instituto de Quimica Rosario (IQUIR-CONICET-UNR), Facultad de Ciencias Bioquimicas y Farmaceuticas, Universidad Nacional de Rosario, Rosario, Argentina; Queen Mary, University of London, Mile End Road, London E1 4NS, UK; and National Center for Natural Products Research & Department of Pharmacology, School of Pharmacy, University of Mississippi, MS 38677, USA. [labadie@iquir-conicet.gov.ar].

16158. **Capes, A., Patterson, S., Wyllie, S., Hallyburton, I., Collie, I. T., McCarroll, A. J., Stevens, M. F., Frearson, J. A., Wyatt, P. G., Fairlamb, A. H. & Gilbert, I. H., 2012.** Quinol derivatives as potential trypanocidal agents. *Bioorganic & Medicinal Chemistry*, **20** (4): 1607-1615.

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

16159. **Carroll, A. R., Nash, B. D., Duffy, S. & Avery, V. M., 2012.** Albopunctatone, an antiplasmodial anthrone-anthraquinone from the Australian *Ascidian Didemnum albopunctatum*. *Journal of Natural Products*, **75** (6): 1206-1209.

School of Environment, Griffith University, Gold Coast, QLD 4222, Australia.

16160. **Costa, T. F., Reis, F. C. & Lima, A. P., 2012.** Substrate inhibition and allosteric regulation by heparan sulphate of *Trypanosoma brucei* cathepsin L. *Biochimica & Biophysica Acta*, **1824** (3): 493-501.

Instituto de Biofísica Carlos Chagas Filho, Universidade Federal do Rio de Janeiro, C.C.S., Ilha do Fundão, Rio de Janeiro, 21949-900, RJ, Brazil. [anapaula@biof.ufrj.br].

16161. **Dalla Rosa, L., Da Silva, A. S., Oliveira, C. B., Brum, I., Benevenutti, E., Dorneles, F., Jaques, J. A., Tavares, K. C., Miletti, L. C., Leal, M. R. & Monteiro, S. G., 2012.** *Trypanosoma evansi*: effects of zinc and copper in experimentally infected rats. *Experimental Parasitology*, **131** (3): 358-362.

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria (UFSM), Predio 20, Sala 4232, Campus Universitario, Camobi, 97105-900 Santa Maria, RS, Brazil.

The aim of this study was to evaluate the effects of a treatment using injectable zinc and copper in rats infected with *Trypanosoma evansi*. 48 rats were divided into eight groups of six animals each. Group A was composed of uninfected animals. Animals from groups B-H were inoculated on the 5th day of the experiment with 1.2x10^{6} trypanosomes. Group B was used as a positive control. The infected groups received prophylactic (C, D and E) and therapeutic (F, G and H) treatments with the zinc and copper, both at a dose of 5 mg/kg^{-1}. The effectiveness of treatment was confirmed by negative blood smears and the polymerase chain reaction (PCR) at the end of the study. All treated animals had their prepatent period and survival prolonged when compared with the control group (group B). Treatment efficacy was 17 percent (C: zinc), 33 percent (D: copper), 50 percent (E: zinc+copper), 0 percent (F: zinc), 50 percent (G: copper) and 50 percent (H: zinc+copper). Thus, we can conclude that treatment with zinc and copper is capable of controlling and/or curing *T. evansi* infection in rats, delaying the parasitaemia and prolonging their survival.

16162. **de Koning, H. P., Gould, M. K., Sterk, G. J., Tenor, H., Kunz, S., Luginbuehl, E. & Seebeck, T., 2012.** Pharmacological validation of *Trypanosoma brucei* phosphodiesterases as novel drug targets. *Journal of Infectious Diseases*, **206** (2): 229-237.

Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, UK.

The development of drugs for neglected infectious diseases often uses parasite-specific enzymes as targets. We here demonstrate that parasite enzymes with highly conserved human homologues may represent a promising reservoir of new potential drug targets. The cyclic nucleotide-specific phosphodiesterases (PDEs) of *Trypanosoma brucei*, causative agent of the fatal human sleeping sickness, are essential for the parasite. The highly conserved human homologues are well established drug targets. We here describe what to our knowledge is the

first pharmacological validation of trypanosomal PDEs as drug targets. High-throughput screening of a proprietary compound library identified a number of potent hits. One compound, the tetrahydrophthalazinone compound A (Cpd A), was further characterized. It causes a dramatic increase of intracellular cyclic adenosine monophosphate (cAMP). Short-term cell viability is not affected, but cell proliferation is inhibited immediately, and cell death occurs within three days. Cpd A prevents cytokinesis, resulting in multinucleated, multiflagellated cells that eventually lyse. These observations pharmacologically validate the highly conserved trypanosomal PDEs as potential drug targets.

16163. **Demoro, B., Sarniguet, C., Sanchez-Delgado, R., Rossi, M., Liebowitz, D., Caruso, F., Olea-Azar, C., Moreno, V., Medeiros, A., Comini, M. A., Otero, L. & Gambino, D., 2012.** New organoruthenium complexes with bioactive thiosemicarbazones as co-ligands: potential anti-trypanosomal agents. *Dalton Transactions*, **41** (5): 1534-1543.

Catedra de Quimica Inorganica, Departamento Estrella Campos, Facultad de Quimica, Universidad de la Republica (UdelaR), Gral. Flores 2124, 11800, Montevideo, Uruguay.

In the search for new therapeutic tools against neglected diseases produced by trypanosomatid parasites, and particularly against African trypanosomiasis, whose aetiological agent is *Trypanosoma brucei*, organoruthenium compounds with bioactive nitrofuran containing thiosemicarbazones (L) as co-ligands were obtained. Four ruthenium (II) complexes with the formula $[Ru_2(p\text{-cymene})_2(L)_2]X_2$, where X = Cl or PF_6, were synthesized and the crystal structures of two of them were solved by X-ray diffraction methods. Two of the complexes showed significant *in vitro* growth inhibition activity against *Trypanosoma brucei brucei* and were highly selective towards trypanosomal cells with respect to mammalian cells (J774 murine macrophages). These promising results make organoruthenium compounds good lead candidates for further developments towards potential antitrypanosomal organometallic drugs.

16164**. Dixit, S. S., Upadhayaya, R. S. & Chattopadhyaya, J., 2012.** New parasite inhibitors encompassing novel conformationally-locked 5'-acyl sulfamoyl adenosines. *Organic & Biomol ecularChemistry,* **10**(30): 6121- 6129.

Program of Chemical Biology, Institute of Cell and Molecular Biology, Biomedical Centre, Uppsala University, SE-75123 Uppsala, Sweden. [jyoti@boc.uu.se].

16165. **Faist, J., Seebacher, W., Saf, R., Brun, R., Kaiser, M. & Weis, R., 2012.** New N-methylpiperazinyl derivatives of bicyclic antiprotozoal compounds. *European Journal of Medicinal Chemistry*, **47** (1): 510-519.

Institute of Pharmaceutical Sciences, Pharmaceutical Chemistry, Karl-Franzens University, Universitatsplatz 1, A-8010 Graz, Austria; Institute for Chemistry and Technology of Materials (ICTM), Graz University of Technology, Stremayrgasse 16, A-8010 Graz, Austria; and Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland. [robert.weis@uni-graz.at].

16166**. Feng, Y., Davis, R. A., Sykes, M. L., Avery, V. M. & Quinn, R. J., 2012.** Iotrochamides A and B, antitrypanosomal compounds from the Australian marine sponge *Iotrochota* sp. *Bioorganic & Medicinal Chemistry Letters,* **22**(14): 4873-4876.

Eskitis Institute, Griffith University, Brisbane, QLD 4111, Australia. [R.Quinn@griffith.edu.au].

Bioassay-guided isolation of the CH_2Cl_2/MeOH extract from the Australian sponge *Iotrochota* sp. resulted in the purification of two new N-cinnamoyl-amino acids, iotrochamides A (1) and B (2). The chemical structures of 1 and 2 were determined by 1D/2D NMR and MS data analyses. Compounds 1 and 2 were shown to inhibit *Trypanosoma brucei brucei* with IC_{50} values of 3.4 and 4.7 µM, respectively.

16167. **Ferreira, L. G. & Andricopulo, A. D., 2012.** Structure- and ligand-based structure-activity relationships for a series of inhibitors of aldolase. *Current Computer Aided Drug Design*. **E publication ahead of print 25 June.**

Laboratorio de Quimica Medicinal e Computacional, Instituto de Fisica de Sao Carlos, Universidade de Sao Paulo, Av. Trabalhador Sao-Carlense 400, 13560-970, Sao Carlos-SP, Brazil. [aandrico@if.sc.usp.br].

16168. **Friedman, A. J., Durrant, J. D., Pierce, L. C., McCorvie, T. J., Timson, D. J. & McCammon, J. A., 2012.** The molecular dynamics of *Trypanosoma brucei* UDP-galactose 4'-epimerase: a drug target for African sleeping sickness. *Chemical Biology & Drug Design,* **80**(2) 173-181.

Biomedical Sciences Graduate Program, University of California San Diego, La Jolla, CA 92093-0365, USA; Department of Chemistry and Biochemistry, University of California San Diego, La Jolla, CA 92093-0365, USA; School of Biological Sciences, Queen's University Belfast, Medical Biology Centre, Belfast BT9 7BL, UK; Department of Chemistry and Biochemistry, NSF Center for Theoretical Biological Physics, National Biomedical Computation Resource, University of California San Diego, La Jolla, CA 92093; USA Department of Pharmacology, University of California San Diego, La Jolla, CA 92093, USA; and Howard Hughes Medical Institute, University of California San Diego, La Jolla, CA 92093, USA. [a1friedm@ucsd.edu].

During the past century, several epidemics of human African trypanosomiasis, a deadly disease caused by the protist *Trypanosoma brucei*, have afflicted sub-Saharan Africa. Over 10 000 new victims are reported each year, with hundreds of thousands more at risk. As current drug treatments are either highly toxic or ineffective, novel trypanocides are urgently needed. The *T. brucei* galactose synthesis pathway is one potential therapeutic target. Although galactose is essential for *T. brucei* survival, the parasite lacks the transporters required to intake galactose from the environment. UDP-galactose 4'-epimerase (TbGalE) is responsible for the epimerization of UDP-glucose to UDP-galactose and is therefore of great interest to medicinal chemists. Using molecular dynamics simulations, we investigated the atomistic motions of TbGalE in both the apo and holo states. The sampled conformations and protein dynamics depend not only on the presence of a UDP-sugar ligand, but also on the chirality of the UDP-sugar C4 atom. This dependence provides important insights into TbGalE function and may help guide future computer-aided drug discovery efforts targeting this protein.

16169. **Fueller, F., Jehle, B., Putzker, K., Lewis, J. D. & Krauth-Siegel, R. L., 2012.** High throughput screening against the peroxidase cascade of African trypanosomes identifies antiparasitic compounds that inactivate tryparedoxin. *Journal of Biological*

Chemistry, **287** (12): 8792-8802.

Biochemie-Zentrum der Universitat Heidelberg, Heidelberg, Germany.

In African trypanosomes, the detoxification of broad spectrum hydroperoxides relies on a unique cascade composed of trypanothione (TSH2), trypanothione reductase, tryparedoxin (Tpx), and nonselenium glutathione peroxidase-type enzymes. All three proteins are essential for *Trypanosoma brucei*. Here, we subjected the complete system to a high throughput screening approach with nearly 80 000 chemicals. Twelve compounds inhibited the peroxidase system. All but one carried chloroalkyl substituents. The detailed kinetic analysis showed that two compounds weakly inhibited trypanothione reductase, but none of them specifically interacted with the peroxidase. They proved to be time-dependent inhibitors of Tpx-modifying Cys-40, the first cysteine of its active site WCPPC motif. Importantly, gel shift assays verified Tpx as a target in the intact parasites. T(SH)(2), present in the *in vitro* assays and in the cells in high molar excess, did not interfere with Tpx inactivation. The compounds inhibited the proliferation of bloodstream *T. brucei* with EC_{50} values down to <1 μM and exerted up to 83-fold lower toxicity toward HeLa cells. Irreversible inhibitors are traditionally regarded as unfavourable. However, a large number of antimicrobials and anticancer therapeutics acts covalently with their target protein. The compounds identified here also interacted with recombinant human thioredoxin, a distant relative of Tpx. This finding might even be exploited for thioredoxin-based anticancer drug development approaches reported recently. The fact that the T(SH)(2)/Tpx couple occupies a central position within the trypanosomal thiol metabolism and delivers electrons also for the synthesis of DNA precursors renders the parasite-specific oxidoreductase an attractive drug target molecule.

16170. **Ganfon, H., Bero, J., Tchinda, A. T., Gbaguidi, F., Gbenou, J., Moudachirou, M., Frederich, M. & Quetin-Leclercq, J., 2012.** Antiparasitic activities of two sesquiterpenic lactones isolated from *Acanthospermum hispidum* D.C. *Journal of Ethnopharmacology*, **141** (1): 411-417.

Pharmacognosy Research Group, Louvain Drug Research Institute, Université catholique de Louvain, Avenue E. Mounier B1.72.03, B-1200 Brussels, Belgium. [pdalsenter@ufpr.br].

16171. **Glans, L., Hu, W., Jost, C., de Kock, C., Smith, P. J., Haukka, M., Bruhn, H., Schatzschneider, U. & Nordlander, E., 2012.** Synthesis and biological activity of cymantrene and cyrhetrene 4-aminoquinoline conjugates against malaria, leishmaniasis, and trypanosomiasis. *Dalton Transactions*, **41** (21): 6443-6450.

Chemical Physics, Center for Chemistry and Chemical Engineering, Lund University, Lund, Sweden.

16172. **Gressler, L. T., Da Silva, A. S., Machado, G., Rosa, L. D., Dorneles, F., Oliveira, M. S., Zanette, R. A., de Vargas, A. C. & Monteiro, S. G., 2012.** Susceptibility of *Trypanosoma evansi* to propolis extract *in vitro* and in experimentally infected rats. *Research in Veterinary Science*. **Available online 9 March.**

Department of Microbiology and Parasitology, Universidade Federal de Santa Maria, Brazil; and Department of Preventive Veterinary Medicine, Universidade Federal de Santa Maria, Brazil [sgmonteiro@uol.com.br].

Current therapy of *Trypanosoma evansi* infections is not effective for the vast majority of animals with relapsing parasitaemia and clinical signs. Recently, attention is being focused on the antiparasitic activity of propolis. This study evaluated the susceptibility of *T. evansi* to propolis extract *in vitro* and *in vivo*. A dose-dependent trypanocidal activity of propolis extract was observed *in vitro*. All trypomastigotes were killed 1h after incubation with 10 µg mL^{-1} of the extract. *In vivo*, concentrations of 100, 200, 300 and 400 mg/kg^{-1} administered orally for 10 consecutive days showed no curative effect, and the rats died from the disease. However, rats treated with the two highest concentrations of propolis extract showed higher longevity than the other groups. Based on these data, we concluded that *T. evansi* is susceptible to propolis *in vitro*. Despite the lack of curative efficacy observed *in vivo* at the concentrations tested, the propolis extract can prolong life in rats infected with the protozoan.

16173. **Hargrove, T. Y., Wawrzak, Z., Liu, J., Waterman, M. R., Nes, W. D. & Lepesheva, G. I., 2012.** Structural complex of sterol 14 alpha-demethylase (CYP51) with 14 alpha-methylenecyclopropyl-delta7-24, 25-dihydrolanosterol. *Journal of Lipid Research*, **53** (2): 311-320.

Department of Biochemistry, School of Medicine, Vanderbilt University, Nashville, TN 37232, USA.

16174. **He, S., Dayton, A., Kuppusamy, P., Werbovetz, K. A. & Drew, M. E., 2012.** Induction of oxidative stress in *Trypanosoma brucei* by the antitrypanosomal dihydroquinoline OSU-40. *Antimicrobial Agents & Chemotherapy*, **56** (5): 2428-2434.

Division of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, The Ohio State University, Columbus, Ohio, USA. [werbovetz.1@osu.edu].

Dihydroquinoline derivative OSU-40 (1-benzyl-1,2-dihydro-2,2,4-trimethylquinolin-6-yl acetate) is selectively potent against *Trypanosma brucei rhodesiense in vitro* (50 percent inhibitory concentration [IC_{50}], 14 nM; selectivity index, 1 700) and has been proposed to cause the formation of reactive oxygen species (ROS) in African trypanosomes. In the present study, we sought to provide further support for the hypothesis that OSU-40 kills trypanosomes through oxidative stress. Inducible RNA interference (RNAi) was applied to downregulate key enzymes in parasite antioxidant defence, including *T. brucei* trypanothione synthetase (TbTryS) and superoxide dismutase B (TbSODB). Both TbTryS RNAi-induced and TbSODB RNAi-induced cells showed impaired growth and increased sensitivity toward OSU-40 by 2.4-fold and 3.4-fold, respectively. Decreased expression of key parasite antioxidant enzymes was thus associated with increased sensitivity to OSU-40, consistent with the hypothesis that OSU-40 acts through oxidative stress. Finally, the dose-dependent formation of free radicals was observed after incubation of *T. brucei* with OSU-40 utilizing electron spin resonance (ESR) spectroscopy. These data support the notion that the mode of antitrypanosomal action for this class of compounds is to induce oxidative stress.

16175. **Herrmann, F., Sporer, F., Tahrani, A. & Wink, M., 2012.** Antitrypanosomal properties of *Panax ginseng* C. A. Meyer: new possibilities for a remarkable traditional drug. *Phototherapy Research*. **E publication ahead of print 4 April**.

Institute of Pharmacy and Molecular Biotechnology, Heidelberg University, Germany. [Florian.Herrmann@t-online.de].

African trypanosomiasis is still a major health problem in many sub-Saharan countries in Africa. We investigated the effects of three preparations of *Panax ginseng, Panax notoginseng*, isolated ginsenosides, and the polyacetylene panaxynol on *Trypanosoma brucei brucei* and the human cancer cell line HeLa. Hexane extracts and the pure panaxynol were toxic and at the same time highly selective against *T. b. brucei*, whereas methanol extracts and 12 isolated ginsenosides were significantly less toxic and showed only weak selectivity. Panaxynol was cytotoxic against *T. b. brucei* at the concentration of 0.01 µg/mL with a selectivity index of 858, superior even to established antitrypanosomal drugs. We suggest that the inhibition of trypanothione reductase, which is only found in trypanosomes, might explain the observed selectivity. The high selectivity together with a cytotoxic concentration in the range of the bioavailability makes panaxynol and other polyacetylenes in general very promising lead compounds for the treatment of African trypanosomiasis.

16176. **Hiltensperger, G., Jones, N. G., Niedermeier, S., Stich, A., Kaiser, M., Jung, J., Puhl, S., Damme, A., Braunschweig, H., Meinel, L., Engstler, M. & Holzgrabe, U., 2012.** Synthesis and structure-activity relationships of new quinolone-type molecules against *Trypanosoma brucei*. *Journal of Medicinal Chemistry*, **55** (6): 2538-2548.

Institut fur Pharmazie and Lebensmittelchemie, Universitat Wuerzburg, Am Hubland, 97074 Wurzburg, Germany. [holzgrab@pharmazie.uni-wuerzburg.de].

Human African trypanosomiasis (HAT) or sleeping sickness is caused by two subspecies of *Trypanosoma brucei*, *Trypanosoma brucei gambiense*, and *Trypanosoma brucei rhodesiense* and is one of Africa's old plagues. It causes a huge number of infections and cases of death per year because, apart from limited access to health services, only inefficient chemotherapy is available. Since it was reported that quinolones such as ciprofloxacin show antitrypanosomal activity, a novel quinolone-type library was synthesized and tested. The biological evaluation illustrated that 4-quinolones with a benzylamide function in position 3 and cyclic or acyclic amines in position 7 exhibit high antitrypanosomal activity. Structure-activity relationships (SAR) are established to identify essential structural elements. This analysis led to lead structure 29, which exhibits promising *in vitro* activity against *T. b. brucei* (IC_{50} = 47 nM) and *T. b. rhodesiense* (IC_{50} = 9 nM) combined with low cytotoxicity against macrophages J774.1. Screening for morphological changes of trypanosomes treated with compounds 19 and 29 suggested differences in the morphology of mitochondria of treated cells compared with those of untreated cells. Segregation of the kinetoplast is hampered in trypanosomes treated with these compounds; however, topoisomerase II is probably not the main drug target.

16177. **Ishiyama, A., Otoguro, K., Iwatsuki, M., Namatame, M., Nishihara-Tsukashima, A., Takahashi, Y., Onodera, H., Yamada, H. & Omura, S., 2012.** *In vitro* antitrypanosomal activity of five low-MW antibiotics. *Journal of Antibiotics (Tokyo)*, **65** (2): 113-114.

Research Center for Tropical Diseases, Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan. [omuras@insti.kitasato-u.ac.jp].

16178. **Johnson, T. A., Sohn, J., Inman, W. D., Estee, S. A., Loveridge, S. T., Vervoort, H. C., Tenney, K., Liu, J., Ang, K. K., Ratnam, J., Bray, W. M., Gassner, N. C., Shen, Y. Y., Lokey, R. S., McKerrow, J. H., Boundy-Mills, K., Nukanto, A.,**

Kanti, A., Julistiono, H., Kardono, L. B., Bjeldanes, L. F. & Crews, P., 2011. Natural product libraries to accelerate the high-throughput discovery of therapeutic leads. *Journal of Natural Products*, **74** (12): 2545-2555.

Department of Nutritional Sciences & Toxicology, University of California, Berkeley, California 94720, USA. [taj_ucb@berkeley.edu].

A high-throughput (HT) paradigm generating LC-MS-UV-ELSD-based natural product libraries to discover compounds with new bioactivities and/or molecular structures is presented. To validate this methodology, an extract of the Indo-Pacific marine sponge *Cacospongia mycofijiensis* was evaluated using assays involving cytoskeletal profiling, tumour cell lines, and parasites. Twelve known compounds were identified including latrunculins (1-4, 10), fijianolides (5, 8, 9), mycothiazole (11), aignopsanes (6, 7), and sacrotride A (13). Compounds 1-5 and 8-11 exhibited bioactivity not previously reported against the parasite *T. brucei*, while 11 showed selectivity for lymphoma (U937) tumour cell lines. Four new compounds were also discovered including aignopsanoic acid B (13), apo-latrunculin T (14), 20-methoxy-fijianolide A (15), and aignopsane ketal (16). Compounds 13 and 16 represent important derivatives of the aignopsane class, 14 exhibited inhibition of *T. brucei* without disrupting microfilament assembly, and 15 demonstrated modest microtubule-stabilizing effects. The use of removable well plate libraries to avoid false positives from extracts enriched with only one or two major metabolites is also discussed. Overall, these results highlight the advantages of applying modern methods in natural products-based research to accelerate the HT discovery of therapeutic leads and/or new molecular structures using LC-MS-UV-ELSD-based libraries.

16179. **Lethu, S., Bosc, D., Mouray, E., Grellier, P. & Dubois, J., 2012.** New protein farnesyltransferase inhibitors in the 3-arylthiophene 2-carboxylic acid series: diversification of the aryl moiety by solid-phase synthesis. *Journal of Enzyme Inhibition & Medicinal Chemistry*. **E publication ahead of print 11 January.**

Institut de Chimie des Substances Naturelles, CNRS, Centre de Recherche de Gif, Gif sur Yvette , France.

16180. **Maser, P., Wittlin, S., Rottmann, M., Wenzler, T., Kaiser, M. & Brun, R., 2012.** Antiparasitic agents: new drugs on the horizon. *Current Opinion in Pharmacology*. **E publication ahead of print 29 May.**

Swiss Tropical and Public Health Institute, 4003 Basel, Switzerland; University of Basel, 4000 Basel, Switzerland.

The need for new drugs against tropical parasites such as *Plasmodium falciparum* and *Trypanosoma brucei* is persistent since problems with resistance and toxicity are jeopardizing the currently available medicines. Public-private partnerships aiming to develop new medicines for malaria and sleeping sickness have, over the past 12 years, brought forward several drug candidates that have entered clinical trials. These are the synthetic peroxide OZ439 and the spiroindolone NITD609 against *P. falciparum*, and fexinidazole and the oxaborole SCYX-7158 against *T. brucei*. A further class with high chemotherapeutic potential is the diamidines, novel members of which may serve as back-up compounds against trypanosomes and other parasites. Thus, finally, new therapeutic agents against malaria and sleeping sickness are within reach.

16181. **Musuyu Muganza, D., Fruth, B. I., Nzunzu Lami, J., Mesia, G. K., Kambu, O. K., Tona, G. L., Cimanga Kanyanga, R., Cos, P., Maes, L., Apers, S. & Pieters, L., 2012.** *In vitro* antiprotozoal and cytotoxic activity of 33 ethnopharmacologically selected medicinal plants from Democratic Republic of Congo. *Journal of Ethnopharmacology*, **141** (1): 301-308.

Faculty of Pharmaceutical Sciences, University of Kinshasa, PO. Box 212, Kinshasa XI, Congo.

The antiprotozoal and cytotoxic activities of the aqueous extracts from 33 medicinal plants used by traditional healers for the treatment of various parasitic diseases and collected after an ethnopharmacological inventory conducted in the Bolongo area, Bandundu province in DR Congo, were evaluated. Decoctions were prepared, lyophilized and evaluated for *in vitro* antiprotozoal activity against *Trypanosoma b. brucei, Trypanosoma cruzi, Leishmania infantum*, and the chloroquine- and pyrimethamine-resistant K1 strain of *Plasmodium falciparum*. Cytotoxicity against MRC-5 cells was included to assess selectivity of activity. Most of the tested extracts exhibited pronounced (IC_{50}</=5 µg/ml) or good (IC_{50}</=10 µg/ml) antiprotozoal activity against one or more of the selected protozoa. A total of 19 plant extracts inhibited *Trypanosoma b. brucei*, especially the extract from *Isolona hexaloba* stem bark (IC_{50}=1.95 µg/ml., SI=16.5); 8 plant extracts were active against *Trypanosoma cruzi*, the extracts from *Enanatia chlorantha* stem bark and *Quassia africana* root bark being the most active with IC_{50} values of 1.87 and 1.88 µg/ml, respectively (SI=3.0 and 3.3, respectively); 8 plant extracts showed activity against *Leishmania infantum*, with extracts from *Napoleona vogelii* stem bark and *Quassia africana* root bark as the most active with IC_{50} values of 5.66 and 5.04 µg/ml (SI=11.3 and 1.2). Finally, 9 plant extracts inhibited *Plasmodium falciparum* K1 with the extracts from *Quassia africana* (root bark and stem bark) being the most active ones with IC_{50} values of 0.46 and 1.27 µg/ml (SI=13.7 and 13.6). Extracts from *Enantia chlorantha* stem bark, *Piptadeniastrum africanum* stem bark and *Quassia africana* root bark were cytotoxic for MRC-5 cells (CC_{50}<10 µg/ml). These results can partly support and justify the traditional use of some of these plant species for the treatment of parasitic diseases.

16182. **Nibret, E. & Wink, M., 2011.** Trypanocidal and cytotoxic effects of 30 Ethiopian medicinal plants. *Zeitschrift für Naturforschung C*, **66** (11-12): 541-546.

Institut fur Pharmazie und Molekulare Biotechnologie, Universitat Heidelberg, Im Neuenheimer Feld 364, D-69120, Heidelberg, Germany. [wink@uni-hd.de].

Trypanocidal and cytotoxic effects of traditionally used medicinal plants of Ethiopia were evaluated. A total of 60 crude plant extracts were prepared from 30 plant species using CH_2Cl_2 and MeOH. The effect upon cell proliferation by the extracts, for both bloodstream forms of *Trypanosoma brucei brucei* and human leukaemia HL-60 cells was assessed using resazurin as vital stain. Of all extracts evaluated against the trypanosomes, the CH_2Cl_2 extracts from five plants showed trypanocidal activity with an IC_{50} value below 20 µg/mL: *Dovyalis abyssinica* (Flacourtiaceae), IC_{50} = 1.4 µg/mL; *Albizia schimperiana* (Fabaceae), IC_{50} = 7.2 µg/mL; *Ocimum urticifolium* (Lamiaceae), IC_{50} = 14.0 µg/mL; *Acokanthera schimperi* (Apocynaceae), IC_{50} = 16.6 µg/mL; and *Chenopodium ambrosioides* (Chenopodiaceae), IC_{50} = 17.1 µg/mL. A pronounced and selective killing of trypanosomes with minimal toxic effect on human cells was exhibited by *Dovyalis abyssinica* (CH_2Cl_2 extract, SI = 125.0; MeOH extract, SI = 57.7) followed by *Albizia schimperiana* (CH_2Cl_2 extract, SI = 31.3) and *Ocimum urticifolium* (MeOH extract, SI = 16.0). In conclusion, the screening of 30 Ethiopian medicinal plants identified three species with good

antitrypanosomal activities and low toxicity towards human cells. *Dovyalis abyssinica* might be a promising candidate for phytotherapy of trypanosomiasis.

16183. **Ochiana, S. O., Gustafson, A., Bland, N. D., Wang, C., Russo, M. J., Campbell, R. K. & Pollastri, M. P., 2012.** Synthesis and evaluation of human phosphodiesterases (PDE) 5 inhibitor analogues as trypanosomal PDE inhibitors. Part 2. Tadalafil analogues. *Bioorganic & Medicinal Chemistry Letters*, **22** (7): 2582-2584.

Northeastern University Department of Chemistry and Chemical Biology, 417 Egan Research Center, 360 Huntington Avenue, Boston, MA 02115, USA; and Marine Biological Laboratory, Josephine Bay Paul Center for Comparative Molecular Biology and Evolution, 7 MBL Street, Woods Hole, MA 02543, USA. [m.pollastri@neu.edu].

16184. **Olmo, E. D., Diaz-Gonzalez, R., Escarcena, R., Carvalho, L., Bustos, L. A., Navarro, M. & Feliciano, A. S., 2012.** Diamine and aminoalcohol derivatives active against *Trypanosoma brucei*. *Bioorganic & Medicinal Chemistry Letters*, **22** (1): 440-443.

Departamento de Quimica Farmaceutica, Facultad de Farmacia - CIETUS, Universidad de Salamanca, Campus Unamuno, E-37007 Salamanca, Spain; and Instituto de Parasitología y Biomedicina Facultad de Farmacia – Lopez Neyra, CSIC, Avda. del Conocimiento, E-18100 Granada, Spain [olmo@usal.es].

Twenty compounds selected as representative members of three series of long-chain 1,2-diamines, 2-amino-1-alkanols and 1-amino-2-alkanols structurally related to dihydrosphingosin, were synthesized and tested *in vitro* for their ability to inhibit the sleeping sickness parasites *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense*. Eight compounds showed EC_{50} values in the submicromolar range, with selectivity indexes up to 39 related to the respective cytotoxicity values for Vero cells. The parasite phenotype detected after treatment with the most potent compounds showed irreversible cell morphology alterations of the flagellar pocket that lead to inhibition of cell growth and parasite death.

16185. **Papadopoulou, M. V., Bloomer, W. D., Rosenzweig, H. S., Chatelain, E., Kaiser, M., Wilkinson, S. R., McKenzie, C. & Ioset, J. R., 2012.** Novel 3-nitro-1H-1,2,4-triazole-based amides and sulphonamides as potential antitrypanosomal agents. *Journal of Medicinal Chemistry*, **55** (11): 5554-5565.

NorthShore University Health System , Evanston, Illinois, USA; Oakton Community College, Des Plaines, Illinois, USA; Drugs for Neglected Diseases initiative (DNDi), Geneva, Switzerland; Parasite Chemotherapy,Swiss Tropical and Public Health Institute, Basel, Switzerland; University of Basel, Basel, Switzerland; and School of Biological & Chemical Sciences, Queen Mary University of London, London, UK. [mpapadopoulou@northshore.org].

A series of novel 3-nitro-1H-1,2,4-triazole-based (and in some cases 2-nitro-1H-imidazole-based) amides and sulphonamides were characterized for their *in vitro* antitrypanosomal and antileishmanial activities as well as mammalian toxicity. Out of 36 compounds tested, 29 (mostly 3-nitro-1H-1,2,4-triazoles) displayed significant activity against *Trypanosoma cruzi* intracellular amastigotes (IC_{50} ranging from 28 nM to 3.72 µM) without concomitant toxicity to L6 host cells (selectivity 66-2 782). Twenty-three of these active compounds were more potent (up to 58-fold) than the reference drug benznidazole, tested in parallel. In addition,

nine nitrotriazoles which were moderately active (0.5 μM </= IC_{50} < 6.0 μM) against *Trypanosoma brucei rhodesiense* trypomastigotes were 5-31-fold more active against bloodstream-form *Trypanosoma brucei brucei* trypomastigotes engineered to overexpress reduced nicotinamide adenine dinucleotide dependent nitroreductase. Finally, three nitrotriazoles displayed a moderate activity against the axenic form of *Leishmania donovani*. Therefore, 3-nitro-1H-1,2,4-triazole-based amides and sulphonamides are potent antitrypanosomal agents.

16186. **Qiao, Z., Wang, Q., Zhang, F., Wang, Z., Bowling, T., Nare, B., Jacobs, R. T., Zhang, J., Ding, D., Liu, Y. & Zhou, H., 2012.** Chalcone-benzoxaborole hybrid molecules as potent antitrypanosomal agents. *Journal of Medicinal Chemistry*, **55** (7): 3553-3557.

School of Pharmacy, Shanghai Jiao Tong University, 200240, Shanghai, China; and SCYNEXIS, Inc., P.O. Box 12878, Research Triangle Park, North Carolina 27709-2878, USA. [hczhou@sjtu.edu.cn].

We report the novel chalcone-benzoxaborole hybrids and their structure-activity relationship against *Trypanosoma brucei* parasites. The 4-NH_2 derivative 29 and 3-OMe derivative 43 were found to have excellent potency. The synergistic 4-NH_2-3-OMe compound 49 showed an IC_{50} of 0.010 μg/mL and resulted in 100 percent survival and zero parasitaemia in a murine infection model, which represents one of the most potent compounds discovered to date from the benzoxaborole class that inhibit *T. brucei* growth.

16187. **Shibata, S., Gillespie, J. R., Ranade, R. M., Koh, C. Y., Kim, J. E., Laydbak, J. U., Zucker, F. H., Hol, W. G., Verlinde, C. L., Buckner, F. S. & Fan, E., 2012.** Urea-based inhibitors of *Trypanosoma brucei* methionyl-tRNA synthetase: selectivity and *in vivo* characterization. *Journal of Medicinal Chemistry,* **55**(14): 6342-6351.

Department of Biochemistry, Department of Chemistry, and Department of Medicine, University of Washington, Seattle, Washington 98195, USA. [fbuckner@uw.edu9].

16188. **Spinks, D., Torrie, L. S., Thompson, S., Harrison, J. R., Frearson, J. A., Read, K. D., Fairlamb, A. H., Wyatt, P. G. & Gilbert, I. H., 2012.** Design, synthesis and biological evaluation of *Trypanosoma brucei* trypanothione synthetase inhibitors. *ChemMedChem*, **7** (1): 95-106.

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

Trypanothione synthetase (TryS) is essential for the survival of the protozoan parasite *Trypanosoma brucei*, which causes human African trypanosomiasis. It is one of only a handful of chemically validated targets for *T. brucei in vivo*. To identify novel inhibitors of TbTryS we screened our in-house diverse compound library that contains 62 000 compounds. This resulted in the identification of six novel hit series of TbTryS inhibitors. Herein we describe the SAR exploration of these hit series, which gave rise to one common series with potency against the enzyme target. Cellular studies on these inhibitors confirmed on-target activity, and the compounds have proven to be very useful tools for further study of the trypanothione pathway in kinetoplastids.

16189. **Steverding, D., Sexton, D. W., Wang, X., Gehrke, S. S., Wagner, G. K. & Caffrey, C. R., 2012.** *Trypanosoma brucei*: Chemical evidence that cathepsin L is essential for survival and a relevant drug target. *International Journal of Parasitology*, **42** (5): 481-488.

Biomedical Research Centre, Norwich Medical School, University of East Anglia, Norwich NR4 7TJ, UK; School of Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK; and Sandler Center for Drug Discovery and Department of Pathology, California Institute for Quantitative Biosciences (QB3), 1700 4th Street, University of California San Francisco, San Francisco, CA 94158, USA. [dsteverding@hotmail.com].

The protozoan parasite causing human African trypanosomiasis, *Trypanosoma brucei*, displays cysteine peptidase activity, the chemical inhibition of which is lethal to the parasite. This activity comprises a cathepsin B (TbCATB) and a cathepsin L (TbCATL). Previous RNA interference (RNAi) data suggest that TbCATB rather than TbCATL is essential to survival even though silencing of the latter was incomplete. Also, chemical evidence supporting the essentiality of either enzyme which would facilitate a target-based drug development programme is lacking. Using specific peptidyl inhibitors and substrates, we quantified the contributions of TbCATB and TbCATL to the survival of *T. brucei*. At 100 µM, the minimal inhibitory concentration that kills all parasites in culture, the non-specific cathepsin inhibitors, benzyloxycarbonyl-phenylalanyl-arginyl-diazomethyl ketone (Z-FA-diazomethyl ketone) and (l-3-trans-propylcarbamoyloxirane-2-carbonyl)-l-isoleucyl-l-proline methyl ester (CA-074Me) inhibited TbCATL and TbCATB by >99 percent. The cathepsin L (CATL)-specific inhibitor, ((2S,3S)-oxirane-2,3-dicarboxylic acid 2-[((S)-1-benzylcarbamoyl-2-phenyl-ethyl)-amide] 3-{[2-(4-hydroxy-phenyl)-ethyl]-amide}) (CAA0225), killed parasites with >99 percent inhibition of TbCATL but only 70 percent inhibition of TbCATB. Conversely, the cathepsin B (CATB)-specific inhibitor, (l-3-trans-propylcarbamoyloxirane-2-carbonyl)-l-isoleucyl-l-proline (CA-074), did not affect survival even though TbCATB inhibition at >95 percent was statistically indistinguishable from the complete inhibition by Z-FA-diazomethyl ketone and CA-074Me. The observed inhibition of TbCATL by CA-074 and CA-074Me was shown to be facilitated by the reducing intracellular environment. All inhibitors, except the CATB-specific inhibitor, CA-074, blockaded lysosomal hydrolysis prior to death. The results suggest that TbCATL, rather than TbCATB, is essential to the survival of *T. brucei* and an appropriate drug target.

16190. **Steverding, D., Wang, X., Potts, B. C. & Palladino, M. A., 2012.** Trypanocidal activity of beta-lactone-gamma-lactam proteasome inhibitors. *Planta Medica*, **78** (2): 131-134.

BioMedical Research Centre, Norwich Medical School, University of East Anglia, Norwich, UK; and Nereus Pharmaceuticals, Inc., San Diego, California, USA. [dsteverding@hotmail.com].

Four beta-lactone- gamma-lactam proteasome inhibitors of natural origin were tested for their trypanocidal activities *in vitro* using culture-adapted bloodstream forms of *Trypanosoma brucei*. All four compounds displayed activities in the nanomolar range. The most trypanocidal compounds with 50 percent growth inhibition (GI_{50}) values of around 3 nM were the bromine and iodine analogues of salinosporamide A, a potent proteasome inhibitor produced by the marine actinomycete *Salinispora tropica*. In general, trypanosomes were

more susceptible to the compounds than were human HL-60 cells. The data support the potential of beta-lactone- gamma-lactam proteasome inhibitors for rational anti-trypanosomal drug development.

16191. **Taladriz, A., Healy, A., Flores Perez, E. J., Herrero Garcia, V., Rios Martinez, C., Alkhaldi, A. A., Eze, A. A., Kaiser, M., de Koning, H. P., Chana, A. & Dardonville, C., 2012.** Synthesis and structure-activity analysis of new phosphonium salts with potent activity against African trypanosomes. *Journal of Medicinal Chemistry*, **55** (6): 2606-2622.

Instituto de Quimica Medica, IQM-CSIC, Juan de la Cierva 3, E-28006 Madrid, Spain; Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK; Swiss Tropical and Public Health Institute, Socinstrasse, 57, CH-4002 Basel, Switzerland; University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland; and Instituto de Química Física "Rocasolano", IQFR-CSIC, Serrano 119, E-28006 Madrid, Spain. [dardonville@iqm.csic.es].

A series of 73 bisphosphonium salts and 10 monophosphonium salt derivatives were synthesized and tested *in vitro* against several wild type and resistant lines of *Trypanosoma brucei* (*T. b. rhodesiense* STIB900, *T. b. brucei* strain 427, TbAT1-KO, and TbB48). More than half of the compounds tested showed a submicromolar EC_{50} activity against these parasites. The compounds did not display any cross-resistance to existing diamidine therapies, such as pentamidine. In most cases, the compounds displayed a good selectivity index versus human cell lines. None of the known *T. b. brucei* drug transporters was required for trypanocidal activity, although some of the bisphosphonium compounds inhibited the low affinity pentamidine transporter. It was found that phosphonium drugs act slowly to clear a trypanosome population but that only a short exposure time is needed for irreversible damage to the cells. A comparative molecular field analysis model (CoMFA) was generated to gain insights into the SAR of this class of compounds, identifying key features for trypanocidal activity.

16192. **Torres, E., Duque, M. D., Lopez-Querol, M., Taylor, M. C., Naesens, L., Ma, C., Pinto, L. H., Sureda, F. X., Kelly, J. M. & Vazquez, S., 2012.** Synthesis of benzopolycyclic cage amines: NMDA receptor antagonist, trypanocidal and antiviral activities. *Bioorganic & Medicinal Chemistry*, **20** (2): 942-948.

Laboratori de Quimica Farmaceutica (Unitat Associada al CSIC), Facultat de Farmacia and Institute of Biomedicine (IBUB), Universitat de Barcelona, Av. Diagonal, 643, Barcelona E-08028, Spain; Unitat de Farmacologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, C./St. Llorenç 21, Reus E-43201, Spain; London School of Hygiene and Tropical Medicine, Department of Infectious and Tropical Diseases, Keppel Street, London WC1E 7HT, UK; Rega Institute for Medical Research, Katolieke Universiteit Leuven, 3000 Leuven, Belgium; and Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208-3500, USA; [svazquez@ub.edu].

The synthesis of several 6,7,8,9,10,11-hexahydro-9-methyl-5,7:9,11-dimethano-5H-benzocyclononen-7-amines is reported. Several of them display low µmolar NMDA receptor antagonist and/or trypanocidal activities. Two compounds are endowed with µmolar anti vesicular stomatitis virus activity, while only one compound shows µmolar anti-influenza

activity. The anti-influenza activity of this compound does not seem to be mediated by blocking of the M2 protein.

16193. **Vincent, I. M., Creek, D. J., Burgess, K., Woods, D. J., Burchmore, R. J. & Barrett, M. P., 2012.** Untargeted metabolomics reveals a lack of synergy between nifurtimox and eflornithine against *Trypanosoma brucei*. *PLoS Neglected Tropical Diseases*, **6** (5): e1618.

The Wellcome Trust Centre for Molecular Parasitology, Institute for Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK; Glasgow Polyomics Facility, University of Glasgow, Glasgow, UK; and Pfizer Animal Health, Pfizer Inc., Kalamazoo, Michigan, USA. [michael.barrett@glasgow.ac.uk].

A non-targeted metabolomics-based approach is presented that enables the study of pathways in response to drug action with the aim of defining the mode of action of trypanocides. Eflornithine, a polyamine pathway inhibitor, and nifurtimox, whose mode of action involves its metabolic activation, are currently used in combination as first line treatment against stage 2, CNS-involved, human African trypanosomiasis (HAT). Drug action was assessed using an LC-MS based non-targeted metabolomics approach. Eflornithine revealed the expected changes to the polyamine pathway as well as several unexpected changes that point to pathways and metabolites not previously described in bloodstream form trypanosomes, including a lack of arginase activity and N-acetylated ornithine and putrescine. Nifurtimox was shown to be converted to a trinitrile metabolite indicative of metabolic activation, as well as inducing changes in levels of metabolites involved in carbohydrate and nucleotide metabolism. However, eflornithine and nifurtimox failed to synergise anti-trypanosomal activity *in vitro*, and the metabolomic changes associated with the combination are the sum of those found in each monotherapy with no indication of additional effects. The study reveals how untargeted metabolomics can yield rapid information on drug targets that could be adapted to any pharmacological situation.

16194. **Watson, C. P., Dogruel, M., Mihoreanu, L., Begley, D. J., Weksler, B. B., Couraud, P. O., Romero, I. A. & Thomas, S. A., 2012.** The transport of nifurtimox, an anti-trypanosomal drug, in an *in vitro* model of the human blood-brain barrier: evidence for involvement of breast cancer resistance protein. *Brain Research*, **1436**: 111-121.

King's College London, Institute of Pharmaceutical Science, Waterloo, London, UK; Weill Medical College of Cornell University, New York, NY, USA; INSERM, U1016, Institut Cochin, Paris, France; CNRS, UMR8104, Paris, France; Université Paris Descartes, Paris, France; and The Open University, Department of Life Sciences, Walton Hall, Milton Keynes, UK [sarah.thomas@kcl.ac.uk].

Human African trypanosomiasis (HAT) is a parasitic disease affecting sub-Saharan Africa. The parasites are able to traverse the blood-brain barrier (BBB), which marks stage 2 (S2) of the disease. Delivery of anti-parasitic drugs across the BBB is key to treating S2 effectively and the difficulty in achieving this goal is likely to be a reason why some drugs require highly intensive treatment regimes to be effective. This study aimed to investigate not only the drug transport mechanisms utilised by nifurtimox at the BBB, but also the impact of nifurtimox-

eflornithine combination therapy (NECT) and other anti-HAT drug combination therapies (CTs) on radiolabelled-nifurtimox delivery in an *in vitro* model of drug accumulation and the human BBB, the hCMEC/D3 cell line. We found that nifurtimox appeared to use several membrane transporters, in particular breast-cancer resistance protein (BCRP), to exit the BBB cells. The addition of eflornithine caused no change in the accumulation of nifurtimox, nor did the addition of clinically relevant doses of the other anti-HAT drugs suramin, nifurtimox or melarsoprol, but a significant increase was observed with the addition of pentamidine. The results provide evidence that anti-HAT drugs are interacting with membrane transporters at the human BBB and suggest that combination with known transport inhibitors could potentially improve their efficacy.

16195. **Wenzler, T., Steinhuber, A., Wittlin, S., Scheurer, C., Brun, R. & Trampuz, A., 2012.** Isothermal microcalorimetry, a new tool to monitor drug action against *Trypanosoma brucei* and *Plasmodium falciparum*. *PLoS Neglected Tropical Diseases*, **6** (6): e1668.

Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Basel, Switzerland; University of Basel, Basel, Switzerland; and Infectious Diseases Research Laboratory, Department of Biomedicine, University Hospital Basel, Basel, Switzerland. [tanja.wenzler@unibas.ch].

Isothermal microcalorimetry is an established tool to measure heat flow of physical, chemical or biological processes. The metabolism of viable cells produces heat, and if sufficient cells are present, their heat production can be assessed by this method. In this study, we investigated the heat flow of two medically important protozoans, *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum*. Heat flow signals obtained for these pathogens allowed us to monitor parasite growth on a real-time basis as the signals correlated with the number of viable cells. To showcase the potential of microcalorimetry for measuring drug action on pathogenic organisms, we tested the method with three antitrypanosomal drugs, melarsoprol, suramin and pentamidine and three antiplasmodial drugs, chloroquine, artemether and dihydroartemisinin, each at two concentrations on the respective parasite. With the real time measurement, inhibition was observed immediately by a reduced heat flow compared with that in untreated control samples. The onset of drug action, the degree of inhibition and the time to death of the parasite culture could conveniently be monitored over several days. Microcalorimetry is a valuable element to be added to the toolbox for drug discovery for protozoal diseases such as human African trypanosomiasis and malaria. The method could probably be adapted to other protozoan parasites, especially those growing extracellularly.

16196. **Wyllie, S., Patterson, S., Stojanovski, L., Simeons, F. R., Norval, S., Kime, R., Read, K. D. & Fairlamb, A. H., 2012.** The anti-trypanosome drug fexinidazole shows potential for treating visceral leishmaniasis. *Science Translational Medicine*, **4** (119): 119re111.

Division of Biological Chemistry and Drug Discovery, Wellcome Trust Biocentre, College of Life Sciences, University of Dundee, Dundee, Scotland, UK. [a.h.fairlamb@dundee.ac.uk9].

Safer and more effective oral drugs are required to treat visceral leishmaniasis, a parasitic disease that kills 50 000 to 60 000 people each year in parts of Asia, Africa, and Latin America. Here, we report that fexinidazole, a drug currently in phase 1 clinical trials for

treating African trypanosomiasis, shows promise for treating visceral leishmaniasis. This 2-substituted 5-nitroimidazole drug is rapidly oxidized *in vivo* in mice, dogs, and humans to sulfoxide and sulfone metabolites. Both metabolites of fexinidazole were active against *Leishmania donovani* amastigotes grown in macrophages, whereas the parent compound was inactive. Pharmacokinetic studies with fexinidazole (200 mg/kg) showed that fexinidazole sulfone achieves blood concentrations in mice above the EC_{99} (effective concentration inhibiting growth by 99 percent) value for at least 24 hours after a single oral dose. A once-daily regimen for five days at this dose resulted in a 98.4 percent suppression of infection in a mouse model of visceral leishmaniasis, equivalent to that seen with the drugs miltefosine and pentostam, which are currently used clinically to treat this tropical disease. In African trypanosomes, the mode of action of nitro drugs involves reductive activation via a NADH (reduced form of nicotinamide adenine dinucleotide)-dependent bacterial-like nitroreductase. Overexpression of the leishmanial homologue of this nitroreductase in *L. donovani* increased sensitivity to fexinidazole by 19-fold, indicating that a similar mechanism is involved in both parasites. These findings illustrate the potential of fexinidazole as an oral drug therapy for treating visceral leishmaniasis.

16197. **Xu, X., Olson, C. L., Engman, D. M. & Ames, J. B., 2012.** ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the calflagin Tb24 flagellar calcium binding protein of *Trypanosoma brucei*. *Biomolecular NMR Assignments*. **Available online 28 July.**

Department of Chemistry, University of California, Davis, CA, 95616, USA.

16198. **Yang, P. Y., Wang, M., He, C. Y. & Yao, S. Q., 2012.** Proteomic profiling and potential cellular target identification of K11777, a clinical cysteine protease inhibitor, in *Trypanosoma brucei*. *Chemical Communications (Cambridge)*, **48** (6): 835-837.

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore. [dbshyc@nus.edu.sg].

16199. **Yang, P. Y., Wang, M., Li, L., Wu, H., He, C. Y. & Yao, S. Q., 2012.** Design, synthesis and biological evaluation of potent azadipeptide nitrile inhibitors and activity-based probes as promising anti-*Trypanosoma brucei* agents. *Chemistry*, **18** (21): 6528-6541.

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore; and Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore. [dbshyc@nus.edu.sg].

Trypanosoma cruzi and *Trypanosoma brucei* are parasites that cause Chagas disease and African sleeping sickness, respectively. There is an urgent need for the development of new drugs against both diseases due to the lack of adequate cures and emerging drug resistance. One promising strategy for the discovery of small-molecule therapeutics against parasitic diseases has been to target the major cysteine proteases such as cruzain for *T. cruzi*, and rhodesain/TbCatB for *T. brucei*. Azadipeptide nitriles belong to a novel class of extremely potent cysteine protease inhibitors against papain-like proteases. We herein report the design, synthesis, and evaluation of a series of azanitrile-containing compounds, most of which were shown to potently inhibit both recombinant cruzain and rhodesain at low nanomolar/picomolar ranges. A strong correlation between the potency of rhodesain inhibition (i.e. target-based screening) and trypanocidal activity (i.e. whole-organism-based

screening) of the compounds was observed. To facilitate detailed studies of this important class of inhibitors, selected hit compounds from our screenings were chemically converted into activity-based probes (ABPs), which were subsequently used for *in situ* proteome profiling and cellular localization studies to further elucidate potential cellular targets (on and off) in both the disease-relevant bloodstream form (BSF) and the insect-residing procyclic form (PCF) of *Trypanosoma brucei*. Overall, the inhibitors presented herein show great promise as a new class of anti-trypanosome agents, which possess better activities than existing drugs. The activity-based probes generated from this study could also serve as valuable tools for parasite-based proteome profiling studies, as well as bioimaging agents for studies of cellular uptake and distribution of these drug candidates. Our studies therefore provide a good starting point for further development of these azanitrile-containing compounds as potential anti-parasitic agents.

16200. **Yang, P. Y., Wang, M., Liu, K., Ngai, M. H., Sheriff, O., Lear, M. J., Sze, S. K., He, C. Y. & Yao, S. Q., 2012.** Parasite-based screening and proteome profiling reveal orlistat, an FDA-approved drug, as a potential anti *Trypanosoma brucei* agent. *Chemistry*, **18** (27): 8403-8413.

Department of Chemistry, National University of Singapore, 3 Science Drive 3, Singapore 117543, Singapore; Department of Biological Sciences, National University of Singapore, 14 Science Drive 4, Singapore 117543, Singapore; and School of Biological Sciences, Nanyang Technological University, 60 Nanyang Drive, Singapore 637551, Singapore. [chmyaosq@nus.edu.sg].

Trypanosoma brucei is a parasite that causes African sleeping sickness in humans and nagana in livestock and is transmitted by the tsetse fly. There is an urgent need for the development of new drugs against African trypanosomiasis due to the lack of vaccines and effective drugs. Orlistat (also called tetrahydrolipstatin or THL) is an FDA-approved antiobesity drug targeting primarily the pancreatic and gastric lipases within the gastrointestinal tract. It shows potential activities against tumours, mycobacteria, and parasites. Herein, we report the synthesis and evaluation of an expanded set of orlistat-like compounds, some of which showed highly potent trypanocidal activities in both the bloodstream form (BSF) and the procyclic form (PCF) of *T. brucei*. Subsequent *in situ* parasite-based proteome profiling was carried out to elucidate potential cellular targets of the drug in both forms. Some newly identified targets were further validated by the labelling of recombinantly expressed enzymes in *Escherichia coli* lysates. Bioimaging experiments with a selected compound were carried out to study the cellular uptake of the drug in *T. brucei*. Results indicated that orlistat is much more efficiently taken up by the BSF than the PCF of *T. brucei* and has clear effects on the morphology of mitochondria, glycosomes, and the endoplasmic reticulum in both BSF and PCF cells. These results support specific effects of orlistat on these organelles and correlate well with our *in situ* proteome profiling. Given the economic challenges of *de novo* drug development for neglected diseases, we hope that our findings will stimulate further research towards the conversion of orlistat-like compounds into new trypanocidal drugs.

16201. **Zhao, Y., Wang, Q., Meng, Q., Ding, D., Yang, H., Gao, G., Li, D., Zhu, W. & Zhou, H., 2012.** Identification of *Trypanosoma brucei* leucyl-tRNA synthetase inhibitors by pharmacophore- and docking-based virtual screening and synthesis. *Bioorganic & Medicinal Chemistry*, **20** (3): 1240-1250.

School of Pharmacy, Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai

200240, China; and Drug Discovery and Design Center, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, 555 Zuchongzhi Road, Shanghai 201203, China. [hczhou@sjtu.edu.cn].

16202. **Zimmermann, S., Kaiser, M., Brun, R., Hamburger, M. & Adams, M., 2012.** Cynaropicrin: the first plant natural product with *in vivo* activity against *Trypanosoma brucei*. *Planta Medica*, **78** (6): 553-556.

Department of Pharmaceutical Sciences, Division of Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, Basel, Switzerland.

A screen of 1 800 plant and fungal extracts with subsequent HPLC-based activity profiling was done to identify new antiprotozoal leads from nature. This led to the identification of cynaropicrin from the herb *Centaurea salmantica* L. (Asteraceae) as a potent *in vitro* inhibitor of *Trypanosoma brucei rhodesiense*. It preferentially inhibited *T. b rhodesiense* (IC_{50} of 0.3 µM) and *T. brucei gambiense* (IC_{50} of 0.2 µM), compared with *Trypanosoma cruzi* (IC_{50} of 4.4 µM) and *Plasmodium falciparum* (IC_{50} of 3.0 µM). Testing against melarsoprol- and pentamidine-resistant strains (IC_{50}'s of 0.3 µM and 0.1 µM, respectively) showed no cross-resistance. Intraperitoneal administration of 2 x 10 mg/kg bodyweight/day in the *T. b. rhodesiense* STIB 900 acute mouse model led to a 92 percent reduction of parasitaemia compared to untreated controls on day seven post-infection. Removal of the 2-hydroxymethyl-2-propenoyl moiety of cynaropicrin led to a loss of toxicity towards *T. b. rhodesiense*. Cytotoxicities against rat myoblasts (L6 cells), human colon adenocarcinoma cells, and murine peritoneal macrophages were measured, and selectivity indices of 7.8, 62, and 9.5 were determined. This is the first report of a plant natural product with potent *in vivo* activity against *Trypanosoma brucei*.

16203. **Zimmermann, S., Thomi, S., Kaiser, M., Hamburger, M. & Adams, M., 2012.** Screening and HPLC-based activity profiling for new antiprotozoal leads from European plants. *Scientia Pharmaceutica*, **80** (1): 205-213.

Department of Pharmaceutical Sciences, Pharmaceutical Biology, University of Basel, Klingelbergstrasse 50, 4056, Basel, Switzerland; and Parasite Chemotherapy, Department of Medical Parasitology and Infection Biology, Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland. [michael.adams@unibas.ch].

Based on a survey of remedies used in Renaissance Europe to treat malaria, we prepared and screened a library of 254 extracts from 61 plants for antiplasmodial activity *in vitro*. HPLC-based activity profiling was performed for targeted identification of active constituents in extracts. One of the most remarkable results was the identification of onopordopicrin, a germacranolide sesquiterpene lactone isolated from *Arctium nemorosum* as a potent inhibitor of *P. falciparum* with an IC_{50} of 6.9 µM. It was tested similarly against *Trypanosoma brucei rhodesiense*, the parasite which causes African sleeping sickness. With an IC_{50} of 0.37 µM, onopordopicrin was one of the most potent natural products reported so far. Cytotoxicity was determined against rat myoblast L6 cells (IC_{50}: 3.06).

8. TRYPANOSOME RESEARCH

(a) CULTIVATION

(b) TAXONOMY, CHARACTERIZATION OF ISOLATES

16204. **Garcia, H. A., Rodrigues, A. C., Martinkovic, F., Minervino, A. H., Campaner, M., Nunes, V. L., Paiva, F., Hamilton, P. B. & Teixeira, M. M., 2011.** Multilocus phylogeographical analysis of *Trypanosoma* (Megatrypanum) genotypes from sympatric cattle and water buffalo populations supports evolutionary host constraint and close phylogenetic relationships with genotypes found in other ruminants. *International Journal of Parasitology*, **41** (13-14): 1385-1396.

Departamento de Parasitologia, Universidade de Sao Paulo, Instituto de Ciencias Biomedicas II, Av. Prof. Lineu Prestes 1374, 05508-900 Sao Paulo, SP, Brazil; Departamento de Patología Veterinaria, Faculdad de Ciencias Veterinarias, Universidad Central de Venezuela, Maracay, Venezuela; Department for Parasitology and Parasitic Diseases, Veterinary Faculty, University of Zagreb, Zagreb, Croatia; Instituto de Biodiversidade e Floresta, Universidade Federal do Oeste do Pará, PA, Brazil; Centro de Ciências Biológicas, Agrárias e da Saúde, Universidade para o Desenvolvimento do Estado e da Região do Pantanal, Campo Grande, MS, Brazil; Departamento de Parasitologia Veterinária, Universidade Federal do Mato Grosso do Sul, MS, Brazil; and Biosciences, College of Life and Environmental Sciences, University of Exeter, UK. [mmgteix@icb.usp.br].

Species of the subgenus *Trypanosoma* (Megatrypanum) have been reported in cattle and other domestic and wild ruminants worldwide. A previous study in Brazil found at least four genotypes infecting cattle (*Bos taurus*), but only one in water buffalo (*Bubalus bubalis*). However, the small number of isolates examined from buffalo, all inhabiting nearby areas, precluded evaluation of their diversity, host associations and geographical structure. To address these questions, we evaluated the genetic diversity and phylogeographical patterns of 25 isolates from water buffalo and 28 from cattle from four separate locations in Brazil and Venezuela. Multigene phylogenetic analyses of ssrRNA, internal transcribed spacer of rDNA (ITSrDNA), 5SrRNA, glycosomal glyceraldehyde 3-phosphate dehydrogenase (gGAPDH), mitochondrial cytochrome b (Cyt b), spliced leader (SL) and cathepsin L-like (CATL) sequences positioned all isolates from sympatric and allopatric buffalo populations into the highly homogeneous genotype TthIA, while the cattle isolates were assigned to three different genotypes, all distinct from TthIA. Polymorphisms in all of these sequences separated the trypanosomes infecting water buffalo, cattle, sheep, antelope and deer, and suggested that they correspond to separate species. Congruent phylogenies inferred with all genes indicated a predominant clonal structure of the genotypes. The multilocus analysis revealed one monophyletic assemblage formed exclusively by trypanosomes of ruminants, which corresponds to the subgenus *T.* (Megatrypanum). The high degree of host specificity, evidenced by genotypes exclusive to each ruminant species and lack of genotype shared by different host species, suggested that the evolutionary history of trypanosomes of this subgenus was strongly constrained by their ruminant hosts. However, incongruence between ruminant and trypanosome phylogenies did not support host-parasite co-evolution, indicating that host switches have occurred across ruminants followed by divergences, giving rise to new trypanosome genotypes adapted exclusively to one host species.

16205. **Verdillo, J. C., Lazaro, J. V., Abes, N. S. & Mingala, C. N., 2012.** Comparative virulence of three *Trypanosoma evansi* isolates from water buffaloes in the Philippines. *Experimental Parasitology*, **130** (2): 130-134.

College of Veterinary Science and Medicine, Central Luzon State University, Science City of Munoz, 3120 Nueva Ecija, Philippines; and Animal Health Unit, Philippine Carabao Center, Science City of Munoz, 3120 Nueva Ecija, Philippines. [cnmingala@hotmail.com].

The virulence of three *Trypanosoma evansi* isolates in Luzon, Visayas and Mindanao water buffaloes was compared by determining the mortality rate, parasitaemia level, clinical signs, and lesions on mice. A total of 51 inbred Balb/c mice (5-6 weeks old) were used and divided into two sets. Set A had three groups corresponding to three trypanosomes isolates (Luzon, Visayas, and Mindanao) with seven mice each whose parasitaemia level, clinical signs, and lesions were noted at necropsy. Set B had three groups corresponding to the three isolates with ten mice each whose mortality was monitored. Each infected mouse was inoculated with 0.2 mL of *T. evansi* intraperitoneally and blood was examined under high power magnification. Their parasitaemia levels were determined using the "rapid matching method". Dead mice were subjected to necropsy and the lungs, liver, spleen, brain and heart were subjected to histopathological processing. Results showed that the mortality rate was highest at day 3 for the Visayas isolates (70 percent), while at day 5 for Luzon (90 percent) and Mindanao (70 percent) isolates. The parasitaemia level of Visayas isolates ($1x10^{8.7}$) reached the earliest peak at day 4 while Luzon isolates ($1x10^{9}$) at day 6 and Mindanao isolates ($1x10^{8.7}$) at day 8. Statistical analysis using least significant difference (LSD) revealed significant difference among treatment means at days 2 and 4. All of the affected mice showed rough hair coat, decreased body weight, and decreased packed cell volume. The most obvious gross lesions observed were pale liver with petechiations and pale muscles. Histopathological examination revealed depletion of the red pulp and extramedullary haematopoiesis in the spleen. Congestion, intralesional trypanosomes in blood vessel and extramedullary haematopoiesis were observed in the liver. In the lungs non-specific lesions observed were pulmonary edema, congestion and haemosiderosis.

16206. **Zidkova, L., Cepicka, I., Szabova, J. & Svobodova, M., 2012.** Biodiversity of avian trypanosomes. *Infection, Genetics & Evolution*, **12** (1): 102-112.

Department of Parasitology, Faculty of Science, Charles University in Prague, Vinicna 7, Prague 128 44, Czech Republic; and Department of Zoology, Faculty of Science, Charles University in Prague, Vinicna 7, Prague 128 44, Czech Republic. [murfar@seznam.cz].

We have studied the biodiversity of trypanosomes from birds and bloodsucking Diptera on a large number of isolates. We used two molecular approaches, random amplification of polymorphic DNA (RAPD) method, and sequence analysis of the small subunit ribosomal RNA (SSU rRNA) gene. The RAPD method divided the isolates into 11 separate lineages. Phylogenetic analysis of the SSU rRNA gene was congruent with the RAPD. Morphometric analysis of kinetoplast width and cell length was in agreement with molecular data. Avian trypanosomes appeared polyphyletic on SSU rDNA tree; thus, they do not represent a taxonomic group. We propose that all lineages recovered by SSU analysis probably represent distinct species of avian trypanosomes. We discuss possible transmission ways and geographical distribution of new avian trypanosome lineages. Finally, we recommend methods that should be used for species determination of avian trypanosomes.

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

[See also **35**: 16042, 16059, 16143, 16146, 16147]

16207. **Achcar, F., Kerkhoven, E. J., Bakker, B. M., Barrett, M. P. & Breitling, R., 2012.** Dynamic modelling under uncertainty: the case of *Trypanosoma brucei* energy metabolism. *PLoS Computational Biology*, **8** (1): e1002352.

Institute of Molecular, Cell and Systems Biology, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK; Groningen Bioinformatics Centre, Groningen Biomolecular Sciences and Biotechnology Institute, University of Groningen, Groningen, The Netherlands; Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK; and Department of Liver, Digestive and Metabolic Diseases, University Medical Centre Groningen, University of Groningen, The Netherlands. [Rainer.Breitling@glasgow.ac.uk].

Kinetic models of metabolism require detailed knowledge of kinetic parameters. However, due to measurement errors or lack of data this knowledge is often uncertain. The model of glycolysis in the parasitic protozoan *Trypanosoma brucei* is a particularly well analysed example of a quantitative metabolic model, but so far it has been studied with a fixed set of parameters only. Here we evaluate the effect of parameter uncertainty. In order to define probability distributions for each parameter, information about the experimental sources and confidence intervals for all parameters were collected. We created a wiki-based website dedicated to the detailed documentation of this information: the SilicoTryp wiki (http://silicotryp.ibls.gla.ac.uk/wiki/Glycolysis). Using information collected in the wiki, we then assigned probability distributions to all parameters of the model. This allowed us to sample sets of alternative models, accurately representing our degree of uncertainty. Some properties of the model, such as the repartition of the glycolytic flux between the glycerol and pyruvate producing branches, are robust to these uncertainties. However, our analysis also allowed us to identify fragilities of the model leading to the accumulation of 3-phosphoglycerate and/or pyruvate. The analysis of the control coefficients revealed the importance of taking into account the uncertainties about the parameters, as the ranking of the reactions can be greatly affected. This work will now form the basis for a comprehensive Bayesian analysis and extension of the model considering alternative topologies.

16208. **Ammerman, M. L., Downey, K. M., Hashimi, H., Fisk, J. C., Tomasello, D. L., Faktorova, D., Kafkova, L., King, T., Lukes, J. & Read, L. K., 2012.** Architecture of the trypanosome RNA editing accessory complex, MRB1. *Nucleic Acids Research*, **40** (12): 5637-5650.

Department of Microbiology and Immunology, School of Medicine, State University of New York at Buffalo, Buffalo, NY 14214, USA; Biology Centre, Institute of Parasitology and Faculty of Science, University of South Bohemia, 37005 Ceske Budejovice (Budweis), Czech Republic. [lread@buffalo.edu].

16209. **Antonenkov, V. D. & Hiltunen, J. K., 2011.** Transfer of metabolites across the peroxisomal membrane. *Biochimica et Biophysica Acta,* **1822**(9):1374-1386.

Department of Biochemistry and Biocenter Oulu, University of Oulu, P.O. Box 3000, FI-90014 Oulu, Finland. [vasily.antonenkov@oulu.fi].

Peroxisomes perform a large variety of metabolic functions that require a constant flow of metabolites across the membranes of these organelles. Over the last few years it has become clear that the transport machinery of the peroxisomal membrane is a unique biological entity since it includes nonselective channels conducting small solutes side by side with transporters for 'bulky' solutes such as ATP. Electrophysiological experiments revealed several channel-forming activities in preparations of plant, mammalian, and yeast peroxisomes and in glycosomes of *Trypanosoma brucei*. The properties of the first discovered peroxisomal membrane channel - mammalian Pxmp2 protein - have also been characterized. The channels are apparently involved in the formation of peroxisomal shuttle systems and in the transmembrane transfer of various water-soluble metabolites including products of peroxisomal beta-oxidation. These products are processed by a large set of peroxisomal enzymes including carnitine acyltransferases, enzymes involved in the synthesis of ketone bodies, thioesterases, and others. This review discusses recent data pertaining to solute permeability and metabolite transport systems in peroxisomal membranes and also addresses mechanisms responsible for the transfer of ATP and cofactors such as an ATP transporter and nudix hydrolases.

16210. **Bagnaresi, P., Nakabashi, M., Thomas, A. P., Reiter, R. J. & Garcia, C. R., 2012.** The role of melatonin in parasite biology. *Molecular & Biochemical Parasitology*, **181** (1): 1-6.

Departamento de Biofisica, Universidade Federal de Sao Paulo, Sao Paulo, Brazil; Departamento de Fisiologia, Instituto de Biociências, Universidade de São Paulo, São Paulo, Brazil; Department of Pharmacology and Physiology, UMDNJ, New Jersey Medical School, Newark, NJ, USA; and Department of Cellular and Structural Biology, The University of Texas Health Science Center at San Antonio, San Antonio, TX, USA. [cgarcia@usp.br].

Regarded as the circadian hormone in mammals, melatonin is a highly conserved molecule, present in nearly all species. In this review, we discuss the role of this indolamine and its precursors in the cell biology of parasites and the role of the molecule in the physiology of the host. In *Plasmodium*, melatonin can modulate intracellular concentrations of calcium and cAMP, which in turn can regulate kinase activity and cell cycle. In *Trypanosoma* infections, modulation of the immune system by melatonin is extremely important in controlling the parasite population. Melatonin also contributes to the inflammatory response to *Toxoplasma gondii* infection. Thus, there are a number of unique adaptations involving intricate connections between melatonin and the biology of the parasite-host relationship.

16211. **Bandini, G., Marino, K., Guther, M. L., Wernimont, A. K., Kuettel, S., Qiu, W., Afzal, S., Kelner, A., Hui, R. & Ferguson, M. A., 2012.** Phosphoglucomutase is absent in *Trypanosoma brucei* and redundantly substituted by phosphomannomutase and phospho-N-acetylglucosamine mutase. *Molecular Microbiology,* **85**(3) 513-534.

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

16212. **Barnes, R. L., Shi, H., Kolev, N. G., Tschudi, C. & Ullu, E., 2012.** Comparative genomics reveals two novel RNAi factors in *Trypanosoma brucei* and provides insight into the core machinery. *PLoS Pathogens*, **8** (5): e1002678.

Department of Internal Medicine, Yale University, New Haven, Connecticut, USA; Division of Epidemiology of Microbial Diseases, School of Public Health, Yale University, New Haven, Connecticut, USA; and Department of Cell Biology, School of Medicine, Yale University, New Haven, Connecticut, USA. [christian.tschudi@yale.edu].

16213. **Benz, C., Clucas, C., Mottram, J. C. & Hammarton, T. C., 2012.** Cytokinesis in bloodstream stage *Trypanosoma brucei* requires a family of katanins and spastin. *PLoS One*, **7** (1): e30367.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, UK. [Tansy.Hammarton@glasgow.ac.uk].

16214. **Bohm, C., Katari, V. S., Brecht, M. & Goringer, H. U., 2012.** *Trypanosoma brucei* 20S editosomes have one RNA substrate-binding site and execute RNA unwinding activity. *Journal of Biological Chemistry,* **287(**31). **E publication ahead of print 1 June.**

Darmstadt University of Technology, Germany.

16215. **Brennand, A., Rigden, D. J. & Michels, P. A., 2012.** Trypanosomes contain two highly different isoforms of peroxin PEX13 involved in glycosome biogenesis. *FEBS Letters*, **586** (13): 1765-1771.

Research Unit for Tropical Diseases, de Duve Institute and Laboratory of Biochemistry, Université catholique de Louvain, Avenue Hippocrate 74, Postal Box B1.74.01, B-1200 Brussels, Belgium. [paul.michels@uclouvain.be].

16216. **Carnes, J., Lewis Ernst, N., Wickham, C., Panicucci, B. & Stuart, K., 2012.** KREX2 is not essential for either procyclic or bloodstream form *Trypanosoma brucei*. *PLoS One*, **7** (3): e33405.

Seattle Biomedical Research Institute, Seattle, Washington, USA. [ken.stuart@sbri.org].

16217. **Castillo-Acosta, V. M., Aguilar-Pereyra, F., Vidal, A. E., Navarro, M., Ruiz-Perez, L. M. & Gonzalez-Pacanowska, D., 2012.** Trypanosomes lacking uracil-DNA glycosylase are hypersensitive to antifolates and present a mutator phenotype. *International Journal of Biochemistry & Cell Biology,* **44**(9): 1455-1468.

Instituto de Parasitologia y Biomedicina "Lopez-Neyra". Consejo Superior de Investigaciones Cientificas. Parque Tecnologico de Ciencias de la Salud, Avenida del Conocimiento, s/n 18100-Armilla (Granada), Spain. [dgonzalez@ipb.csic.es].

Cells contain low amounts of uracil in DNA which can be the result of dUTP misincorporation during replication or cytosine deamination. Elimination of uracil in the base

excision repair pathway yields an abasic site, which is potentially mutagenic unless repaired. The *Trypanosoma brucei* genome presents a single uracil-DNA glycosylase responsible for removal of uracil from DNA. Here we establish that no excision activity is detected on U:G, U:A pairs or single-strand uracil-containing DNA in uracil-DNA glycosylase null mutant cell extracts, indicating the absence of back-up uracil excision activities. While procyclic forms can survive with moderate amounts of uracil in DNA, an analysis of the mutation rate and spectra in mutant cells revealed a hypermutator phenotype where the predominant events were GC to AT transitions and insertions. Defective elimination of uracil via the base excision repair pathway gives rise to hypersensitivity to antifolates and oxidative stress and an increased number of DNA strand breaks, suggesting the activation of alternative DNA repair pathways. Finally, we show that uracil-DNA glycosylase defective cells exhibit reduced infectivity *in vivo* demonstrating that efficient uracil elimination is important for survival within the mammalian host.

16218. **Charret, K. S., Requena, C. E., Castillo-Acosta, V. M., Ruiz-Perez, L. M., Gonzalez-Pacanowska, D. & Vidal, A. E., 2012.** *Trypanosoma brucei* AP endonuclease 1 has a major role in the repair of abasic sites and protection against DNA-damaging agents. *DNA Repair (Amst)*, **11** (1): 53-64.

Instituto de Parasitologia y Biomedicina Lopez-Neyra, Consejo Superior de Investigaciones Cientificas (CSIC), Armilla, Granada, Spain. [avidal@ipb.csic.es].

DNA repair mechanisms guarantee the maintenance of genome integrity, which is critical for cell viability and proliferation in all organisms. As part of the cellular defences to DNA damage, apurinic/apyrimidinic (AP) endonucleases repair the abasic sites produced by spontaneous hydrolysis, oxidative or alkylation base damage and during base excision repair (BER). *Trypanosoma brucei*, the protozoan pathogen responsible of human sleeping sickness, has a class II AP endonuclease (TBAPE1) with a high degree of homology to human APE1 and bacterial exonuclease III. The purified recombinant enzyme cleaves AP sites and removes 3'-phosphoglycolate groups from 3'-ends. To study its cellular function, we have established TBAPE1-deficient cell lines derived from bloodstream stage trypanosomes, thus confirming that the AP endonuclease is not essential for viability in this cell type under *in vitro* culture conditions. The role of TBAPE1 in the removal of AP sites is supported by the inverse correlation between the level of AP endonuclease in the cell and the number of endogenously generated abasic sites in its genomic DNA. Furthermore, depletion of TBAPE1 renders cells hypersensitive to AP site and strand break-inducing agents such as methotrexate and phleomycin respectively but not to alkylating agents. Finally, the increased susceptibility that TBAPE1-depleted cells show to nitric oxide suggests an essential role for this DNA repair enzyme in protection against the immune defences of the mammalian host.

16219. **Choi, J. & El-Sayed, N. M., 2012.** Functional genomics of trypanosomatids. *Parasite Immunology*, **34** (2-3): 72-79.

Department of Cell Biology and Molecular Genetics, University of Maryland, College Park, MD 20742, USA. [elsayed@umd.edu].

The decoding of the Tritryp reference genomes nearly seven years ago provided a first peek into the biology of pathogenic trypanosomatids and a blueprint that has paved the way for genome-wide studies. Although 60-70 percent of the predicted protein coding genes in *Trypanosoma brucei*, *Trypanosoma cruzi* and *Leishmania major* remain unannotated, the functional genomics landscape is rapidly changing. Facilitated by the advent of next-

generation sequencing technologies, improved structural and functional annotation and genes and their products are emerging. Information is also growing for the interactions between cellular components as transcriptomes, regulatory networks and metabolomes are characterized, ushering in a new era of systems biology. Simultaneously, the launch of comparative sequencing of multiple strains of kinetoplastids will finally lead to the investigation of a vast, yet to be explored, evolutionary and pathogenomic space.

16220. **Ciganda, M. & Williams, N., 2012.** Characterization of a novel association between two trypanosome-specific proteins and 5S rRNA. *PLoS One*, **7** (1): e30029.

Department of Microbiology and Immunology & Witebsky Center for Microbial Pathogenesis and Immunology, University at Buffalo, Buffalo, New York, USA. [nw1@buffalo.edu].

16221. **Concepcion-Acevedo, J., Luo, J. & Klingbeil, M. M., 2012.** Dynamic localization of *Trypanosoma brucei* mitochondrial DNA polymerase ID. *Eukaryotic Cell*, **11** (7): 844-855.

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

16222. **Dacheux, D., Landrein, N., Thonnus, M., Gilbert, G., Sahin, A., Wodrich, H., Robinson, D. R. & Bonhivers, M., 2012.** A MAP6-related protein is present in protozoa and is involved in flagellum motility. *PLoS One*, **7** (2): e31344.

Microbiologie Fondamentale et Pathogénicité, Université de Bordeaux, UMR 5234, Bordeaux, France. [melanie.bonhivers@u-bordeaux2.fr].

16223. **Daithankar, V. N., Wang, W., Trujillo, J. R. & Thorpe, C., 2012.** Flavin-linked Erv-family sulfhydryl oxidases release superoxide anion during catalytic turnover. *Biochemistry*, **51** (1): 265-272.

Department of Chemistry and Biochemistry, University of Delaware, Newark, Delaware 19716-2522, USA. [cthorpe@udel.edu].

16224. **D'Archivio, S., Medina, M., Cosson, A., Chamond, N., Rotureau, B., Minoprio, P. & Goyard, S., 2011.** Genetic engineering of *Trypanosoma* (Dutonella) *vivax* and *in vitro* differentiation under axenic conditions. *PLoS Neglected Tropical Diseases*, **5** (12): e1461.

Laboratoire des Processus Infectieux à Trypanosoma, Department of Infection and Epidemiology, Paris, France; Laboratoire de Cristallographie et RMN Biologiques - Université Paris Descartes France; CNRS UMR 8015, Paris, France; and Unité de Biologie Cellulaire des Trypanosomes, CNRS URA 2581, Department of Parasitology, Paris, France. [paola.minoprio@pasteur.fr].

Trypanosoma vivax is one of the most common parasites responsible for animal trypanosomosis, and although this disease is widespread in Africa and Latin America, very few studies have been conducted on the parasite's biology. This is in part due to the fact that no reproducible experimental methods had been developed to maintain the different evolutionary forms of this trypanosome under laboratory conditions. Appropriate protocols

were developed in the 1990s for the axenic maintenance of three major animal *Trypanosoma s*pecies: *T. b. brucei*, *T. congolense* and *T. vivax*. These pioneer studies rapidly led to the successful genetic manipulation of *T. b. brucei* and *T. congolense*. Advances were made in the understanding of these parasites' biology and virulence, and new drug targets were identified. By contrast, challenging *in vitro* conditions have been developed for *T. vivax* in the past, and this per se has contributed to defer both its genetic manipulation and subsequent gene function studies. Here we report on the optimization of non-infective *T. vivax* epimastigote axenic cultures and on the process of parasite *in vitro* differentiation into metacyclic infective forms. We have also constructed the first *T. vivax* specific expression vector that drives constitutive expression of the luciferase reporter gene. This vector was then used to establish and optimize epimastigote transfection. We then developed highly reproducible conditions that can be used to obtain and select stably transfected mutants that continue metacyclogenesis and are infectious in immunocompetent rodents.

16225. **Demir, O. & Amaro, R. E., 2012.** Elements of nucleotide specificity in the *Trypanosoma brucei* mitochondrial RNA editing enzyme RET2. *Journal of Chemical Information & Modeling*, **52** (5): 1308-1318.

Department of Chemistry and Biochemistry, University of California , San Diego, 3234 Urey Hall, 9500 Gilman Drive, MC-0340 La Jolla, California 92093-0332, USA. [ramaro@ucsd.edu].

16226. **Docampo, R. & Lukes, J., 2012.** Trypanosomes and the solution to a 50-year mitochondrial calcium mystery. *Trends in Parasitology*, **28** (1): 31-37.

Center for Tropical and Emerging Global Diseases and Department of Cellular Biology, University of Georgia, Athens, GA 30620, USA. [rdocampo@uga.edu].

The ability of mitochondria to take up Ca^{2+} was discovered 50 years ago. This calcium uptake, through a mitochondrial calcium uniporter (MCU), is important not only for the regulation of cellular ATP concentration but also for more complex pathways such as shaping Ca^{2+} signals and the activation of programmed cell death. The molecular nature of the uniporter remained unknown for decades. By a comparative study of mitochondrial protein profiles of organisms lacking or possessing MCU, such as yeast in the former case and vertebrates and trypanosomes in the latter, two groups recently found the protein that possesses all the characteristics of the MCU. These results add another success story to the already substantial contributions of trypanosomes to mammalian biochemistry.

16227. **DuBois, K. N., Alsford, S., Holden, J. M., Buisson, J., Swiderski, M., Bart, J. M., Ratushny, A. V., Wan, Y., Bastin, P., Barry, J. D., Navarro, M., Horn, D., Aitchison, J. D., Rout, M. P. & Field, M. C., 2012.** NUP-1 is a large coiled-coil nucleoskeletal protein in trypanosomes with lamin-like functions. *PLoS Biology*, **10** (3): e1001287.

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

16228. **Echeverry, M. C., Bot, C., Obado, S. O., Taylor, M. C. & Kelly, J. M., 2012.** Centromere-associated repeat arrays on *Trypanosoma brucei* chromosomes are much more extensive than predicted. *BMC Genomics*, **13**: 29.

Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, London, UK. [john.kelly@lshtm.ac.uk].

16229. **Flinner, N., Schleiff, E. & Mirus, O., 2012.** Identification of two voltage-dependent anion channel-like protein sequences conserved in Kinetoplastida. *Biology Letters*, **8** (3): 446-449.

Department of Biosciences, Molecular Cell Biology of Plants, Goethe University Frankfurt, Frankfurt, Hesse, Germany. [o.mirus@bio.uni-frankfurt.de].

16230. **Frasch, A. P., Carmona, A. K., Juliano, L., Cazzulo, J. J. & Niemirowicz, G. T., 2012.** Characterization of the M32 metallocarboxypeptidase of *Trypanosoma brucei*: differences and similarities with its orthologue in *Trypanosoma cruzi*. *Molecular & Biochemical Parasitology*, **184** (2): 63-70.

Instituto de Investigaciones Biotecnologicas "Dr. Rodolfo Ugalde"-Instituto Tecnologico Chascomus, UNSAM-CONICET, Campus Miguelete, Av. 25 de Mayo y Francia, 1650 San Martin, Buenos Aires, Argentina. [gniemiro@iibintech.com.ar].

Metallocarboxypeptidases (MCP) of the M32 family of peptidases have been identified in a number of prokaryotic organisms but they are absent from eukaryotic genomes with the remarkable exception of those of trypanosomatids. The genome of *Trypanosoma brucei*, the causative agent of sleeping sickness, encodes one such MCP which displays 72 percent identity to the characterized TcMCP-1 from *Trypanosoma cruzi*. As its orthologue, TcMCP-1, *Trypanosoma brucei* MCP is a cytosolic enzyme expressed in both major stages of the parasite. Purified recombinant TbMCP-1 exhibits a significant hydrolytic activity against the carboxypeptidase B substrate FA (furylacryloil)-Ala-Lys at pH 7.0-7.8 resembling the *T. cruzi* enzyme. Several divalent cations had little effect on TbMCP-1 activity but increasing amounts of Co^{2+} inhibited the enzyme. Despite having similar tertiary structure, both protozoan MCPs display different substrate specificity with respect to P1 position. Thus, TcMCP-1 enzyme cleaved Abz-FVK-(Dnp)-OH substrate (where Abz: o-aminobenzoic acid and Dnp: 2,4-dinitrophenyl) whereas TbMCP-1 had no activity on this substrate. Comparative homology models and sequence alignments using TcMCP-1 as a template led us to map several residues that could explain this difference. To verify this hypothesis, site-directed mutagenesis was undertaken replacing the TbMCP-1 residues by those present in TcMCP-1. We found that the substitution A414M led TbMCP-1 to gain activity on Abz-FVK-(Dnp)-OH, thus showing that this residue is involved in specificity determination, probably being part of the S1 sub-site. Moreover, the activity of both protozoan MCPs was explored on two vasoactive compounds such as bradykinin and angiotensin I resulting in two different hydrolysis patterns.

16231. **Gannavaram, S. & Debrabant, A., 2012.** Involvement of TatD nuclease during programmed cell death in the protozoan parasite *Trypanosoma brucei*. *Molecular Microbiology*, **83** (5): 926-935.

Laboratory of Emerging Pathogens, Division of Emerging and Transfusion Transmitted Diseases, Center for Biologics Evaluation and Research, US Food and Drug Administration, Bethesda, MD 20892, USA.

In this report, we describe the involvement of TatD nuclease during programmed cell death (PCD) in the human protozoan parasite *Trypanosoma brucei*. *T. brucei* TatD nuclease showed

intrinsic DNase activity, was localized in the cytoplasm and translocated to the nucleus when cells were treated with inducers previously demonstrated to cause PCD in *T. brucei*. Overexpression of TatD nuclease resulted in elevated PCD and conversely, loss of TatD expression by RNAi conferred significant resistance to the induction of PCD in *T. brucei*. Co-immunoprecipitation studies revealed that TatD nuclease interacts with endonucleaseG suggesting that these two nucleases could form a DNA degradation complex in the nucleus. Together, biochemical activity, RNAi and subcellular localization results demonstrate the role of TatD nuclease activity in DNA degradation during PCD in these evolutionarily ancient eukaryotic organisms. Further, in conjunction with endonucleaseG, TatD may represent a critical nuclease in a caspase-independent PCD pathway in trypanosomatid parasites since caspases have not been identified in these organisms.

16232. **Gibson, W., 2012.** The origins of the trypanosome genome strains *Trypanosoma brucei brucei* TREU 927, *T. b. gambiense* DAL 972, *T. vivax* Y486 and *T. congolense* IL3000. *Parasites & Vectors*, **5**: 71.

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

The genomes of several tsetse-transmitted African trypanosomes (*Trypanosoma brucei brucei, T. b. gambiense, T. vivax, T. congolense*) have been sequenced and are available to search online. The trypanosome strains chosen for the genome sequencing projects were selected because they had been well characterised in the laboratory, but all were isolated several decades ago. The purpose of this short review is to provide some background information on the origins and biological characterisation of these strains as a source of reference for future users of the genome data. With high throughput sequencing of many more trypanosome genomes in prospect, it is important to understand the phylogenetic relationships of the genome strains.

16233. **Glover, L. & Horn, D., 2012.** Trypanosomal histone gammaH2A and the DNA damage response. *Molecular & Biochemical Parasitology*, **183** (1): 78-83.

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

DNA damage and repair in trypanosomatids impacts virulence, drug resistance and antigenic variation but, currently, little is known about DNA damage responses or cell cycle checkpoints in these divergent protozoa. One of the earliest markers of DNA damage in eukaryotes is gammaH_2A(X), a serine phosphorylated histone H_2A (variant). Here, we report the identification and initial characterization of gammaH_2A in *Trypanosoma brucei*. We identified Thr^{130} within the replication-dependent histone H_2A as a candidate phosphorylation site and found that the abundance of this trypanosomal gammaH_2A increased *in vivo* in response to DNA damage. Nuclear gammaH_2A foci mark the sites of putative natural replication fork stalling, sites of meganuclease-induced DNA double strand breaks and sites of methyl methanesulphonate-induced DNA damage. Naturally occurring and meganuclease-induced gammaH_2A and RAD_{51} double-positive repair foci are typically found in S-phase or G_2 nuclei. The results link trypanosomal gammaH_2A, with an unusual histone modification motif, to DNA damage sensing and mitotic checkpoint signalling.

16234. **Gnipova, A., Panicucci, B., Paris, Z., Verner, Z., Horvath, A., Lukes, J. & Zikova, A., 2012.** Disparate phenotypic effects from the knockdown of various *Trypanosoma*

brucei cytochrome c oxidase subunits. *Molecular & Biochemical Parasitology*, **184** (2): 90-98.

Institute of Parasitology, Biology Centre, Ceske Budejovice, Czech Republic; Faculty of Science, Comenius University, Bratislava, Slovakia. [azikova@paru.cas.cz].

16235. **Gonzalez-Salgado, A., Steinmann, M. E., Greganova, E., Rauch, M., Maser, P., Sigel, E. & Butikofer, P., 2012.** Myo-inositol uptake is essential for bulk inositol phospholipid but not glycosylphosphatidylinositol synthesis in *Trypanosoma brucei*. *Journal of Biological Chemistry*, **287** (16): 13313-13323.

Institute of Biochemistry and Molecular Medicine, University of Bern, Buhlstrasse 28, 3012 Bern, Switzerland.

Myo-inositol is an essential precursor for the production of inositol phosphates and inositol phospholipids in all eukaryotes. Intracellular myo-inositol is generated by *de novo* synthesis from glucose 6-phosphate or is provided from the environment via myo-inositol symporters. We show that in *Trypanosoma brucei*, the causative pathogen of human African sleeping sickness and nagana in domestic animals, myo-inositol is taken up via a specific proton-coupled electrogenic symport and that this transport is essential for parasite survival in culture. Down-regulation of the myo-inositol transporter using RNA interference inhibited uptake of myo-inositol and blocked the synthesis of the myo-inositol-containing phospholipids, phosphatidylinositol and inositol phosphorylceramide; in contrast, it had no effect on glycosylphosphatidylinositol production. This together with the unexpected localization of the myo-inositol transporter in both the plasma membrane and the Golgi demonstrates that metabolism of endogenous and exogenous myo-inositol in *T. brucei* is strictly segregated.

16236. **Goringer, H. U., 2012.** Parasite-specific aptamers as biosynthetic reagents and potential pharmaceuticals. *Trends in Parasitology*, **28** (3): 106-113.

Genetics, Darmstadt University of Technology, Schnittspahnstrasse 10, 64287 Darmstadt, Germany. [goringer@hrzpub.tu-darmstadt.de].

Aptamers are short, synthetic nucleic acid molecules. They are generated by a Darwinian-type *in vitro* evolution method known as "systematic evolution of ligands by exponential enrichment" (SELEX). SELEX represents an experimental platform to identify rare ligands with predetermined functionality from combinatorial nucleic acid libraries. Since its discovery about 20 years ago the method has been instrumental in identifying a large number of aptamers that recognize targets of very different chemistry and molecular complexity. Although aptamers have been converted into sophisticated biomolecular tools for a diverse set of technologies, only a limited number of aptamers have been selected as binding reagents for parasites or parasite-derived molecules. Here the published examples of aptamers that target *Leishmania*-, *Trypanosoma*- and *Plasmodia*-specific molecules are reviewed.

16237. **Greganova, E. & Butikofer, P., 2012.** Ethanolamine phosphoglycerol attachment to eEF1A is not essential for normal growth of *Trypanosoma brucei*. *Scientific Reports*, **2**: 254.

Swiss Tropical and Public Health Institute, Socinstrasse 57, 4002 Basel, Switzerland. [peter.buetikofer@ibmm.unibe.ch].

16238. **Guo, X., Carnes, J., Ernst, N. L., Winkler, M. & Stuart, K., 2012.** KREPB6, KREPB7, and KREPB8 are important for editing endonuclease function in *Trypanosoma brucei*. *RNA*, **18** (2): 308-320.

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

16239. **Haanstra, J. R., van Tuijl, A., van Dam, J., van Winden, W., Tielens, A. G., van Hellemond, J. J. & Bakker, B. M., 2012.** Proliferating bloodstream-form *Trypanosoma brucei* use a negligible part of consumed glucose for anabolic processes. *International Journal of Parasitology*, **42** (7): 667-673.

Department of Molecular Cell Physiology, Faculty of Earth and Life Sciences, Vrije Universiteit Amsterdam, De Boelelaan 1085, NL-1081 HV Amsterdam, The Netherlands; and Department of Paediatrics, Centre for Liver, Digestive and Metabolic Diseases, University of Groningen, University Medical Centre Groningen, Hanzeplein 1, NL-9713 GZ Groningen, The Netherlands. [B.M.Bakker@med.umcg.nl].

16240. **Hamilton, P. B., 2012.** Is *Trypanosoma vivax* genetically diverse? *Trends in Parasitology*, **28** (5): 173.

Biosciences, College of Life and Environmental Sciences, University of Exeter, Exeter, EX4 4QD, UK. [p.b.hamilton@exeter.ac.uk].

In their recent review on diversity of African tsetse fly transmitted trypanosomes, Tait et al. suggest that genetic diversity of *Trypanosoma vivax*, a major pathogen of African and South American cattle, is limited. This is primarily based on DNA microsatellite analysis of 31 *T. vivax* isolates from a single area in The Gambia. However, this assessment ignores evidence from several studies that have used isoenzymes, DNA microsatellites and more recently sequence data that have revealed high levels of genetic diversity within this species, particularly in East Africa. A partial *T. vivax* 18S rDNA sequence, obtained from an infected tsetse fly in Tanzania had greatest similarity to that of a West African *T. vivax* isolate, yet diverged by 14 percent; two novel genotypes were discovered in wild antelope from Mozambique, one of which caused severe disease in a goat , and three diverse genotypes were discovered in tsetse flies from Tanzania. Indeed, analysis of glycosomal glyceraldehyde phosphate dehydrogenase (gGAPDH) gene sequences suggests that *T. vivax* diversity is similar to that of *Trypanosoma congolense*, a species where the different strains (forest, savannah and kilifi) arguably represent different species. The limited genetic diversity found in The Gambia reflects the results of a study which found isolates with identical gGAPDH gene sequences from across West Africa (The Gambia, Nigeria and Cameroon) and South America. By contrast, considerable polymorphism (11 distinct alleles) was identified in 31 *T. vivax* infections in field collected tsetse flies from Cameroon, using primers targeting a region that contains a microsatellite sequence. The results from these studies are not directly comparable, as they used different genotyping techniques; DNA microsatellites evolve quickly, whereas the gGAPDH gene has a slow evolutionary rate and is generally used for resolving species-level relationships. It is therefore difficult to assess the true genetic diversity of *T. vivax* in West Africa, particularly as *T. vivax* has not been extensively sampled from wild mammals, tsetse flies and other biting flies in the region, which could potentially harbour greater diversity. Additionally, the primer sets used for initial identification in the study of Duffy et al. lack the ability to detect divergent *T. vivax* genotypes , and this may also be the case for the primer sets used for microsatellite genotyping that were designed from genomic

sequences from a West African strain . By contrast, the studies that have revealed the diverse genotypes in East Africa have used "generic" primers, designed to amplify DNA from a wide range of trypanosome species, and therefore may be more likely to pick up novel genotypes. It is clear that our knowledge of the diversity of *T. vivax* is limited. Understanding the true diversity of this important parasite and its significance in terms of diagnosis, disease, differential drug response and the evolution of resistance to chemotherapeutic treatments will require broader surveys and improved genotyping techniques.

16241. **Han, J., Miranda-Saavedra, D., Luebbering, N., Singh, A., Sibbet, G., Ferguson, M. A. & Cleghon, V., 2012.** Deep evolutionary conservation of an intramolecular protein kinase activation mechanism. *PLoS One*, **7** (1): e29702.

Division of Developmental Biology, Cincinnati Children's Hospital Medical Center, Cincinnati, Ohio, USA. [vaughn.cleghon@cchmc.org].

16242. **Hoog, J. L., Bouchet-Marquis, C., McIntosh, J. R., Hoenger, A. & Gull, K., 2012.** Cryo-electron tomography and 3-D analysis of the intact flagellum in *Trypanosoma brucei*. *Journal of Structural Biology*, **178** (2): 189-198.

The Boulder Laboratory for 3-D Electron Microscopy of Cells, MCD-Biology, University of Colorado at Boulder, Boulder, CO 80309-0347, USA. [hoog@colorado.edu].

16243. **Hu, H., Hu, L., Yu, Z., Chasse, A. E., Chu, F. & Li, Z., 2012.** An orphan kinesin in trypanosomes cooperates with a kinetoplastid-specific kinesin to maintain cell morphology through regulating subpellicular microtubules. *Journal of Cell Science*. **Advance online publication 23 May.**

Department of Microbiology & Molecular Genetics, University of Texas Medical School at Houston, TX 77030, USA.

16244. **Hu, L., Hu, H. & Li, Z., 2012.** A kinetoplastid-specific kinesin is required for cytokinesis and for maintenance of cell morphology in *Trypanosoma brucei*. *Molecular Microbiology*, **83** (3): 565-578.

Department of Microbiology & Molecular Genetics, University of Texas Medical School at Houston, TX 77030, USA.

Kinesins are motor-based transport proteins that play diverse roles in various cellular processes. The trypanosome genome lacks the homologues of many conserved mitotic kinesins, but encodes a number of trypanosome-specific kinesins with unknown function. Here, we report the biochemical and functional characterization of TbKIN-C, a trypanosome-specific kinesin, which was initially identified through an RNAi screen for cytokinesis genes in *T. brucei*. TbKIN-C possesses *in vitro* ATPase activity and associates with cytoskeletal tubulin microtubules *in vivo*. It is distributed throughout the cytoskeleton with a focal enrichment at the posterior end of the cell during early cell cycle stages. RNAi of TbKIN-C resulted in distorted cell shape with an elongated posterior filled with tyrosinated tubulin microtubules. Silencing of TbKIN-C impaired the segregation of organelles and cytoskeletal structures and led to detachment of the new flagellum and a small portion of the cytoplasm. We also show that RNAi of TbKIN-C compromised cytokinesis and abolished the trans-localization of TbCPC1, a subunit of the chromosomal passenger complex, from the central

spindle to the initiation site of cytokinesis. Our results suggest an essential role of TbKIN-C in maintaining cell morphology, likely through regulating microtubule dynamics at the posterior end of the cell.

16245. **Hughes, L. C., Ralston, K. S., Hill, K. L. & Zhou, Z. H., 2012.** Three-dimensional structure of the trypanosome flagellum suggests that the paraflagellar rod functions as a biomechanical spring. *PLoS One*, **7** (1): e25700.

Department of Microbiology, Immunology and Molecular Genetics, University of California, Los Angeles, Los Angeles, California, USA. [kenthill@mednet.ucla.edu].

Flagellum motility is critical for normal human development and for transmission of pathogenic protozoa that cause tremendous human suffering worldwide. Biophysical principles underlying motility of eukaryotic flagella are conserved from protists to vertebrates. However, individual cells exhibit diverse waveforms that depend on cell-specific elaborations on basic flagellum architecture. *Trypanosoma brucei* is a uniflagellated protozoan parasite that causes African sleeping sickness. The *T. brucei* flagellum is comprised of a 9+2 axoneme and an extra-axonemal paraflagellar rod (PFR), but the three-dimensional (3D) arrangement of the underlying structural units is poorly defined. Here, we use dual-axis electron tomography to determine the 3D architecture of the *T. brucei* flagellum. We define the *T. brucei* axonemal repeating unit. We observe direct connections between the PFR and axonemal dyneins, suggesting a mechanism by which mechanochemical signals may be transmitted from the PFR to axonemal dyneins. We find that the PFR itself is comprised of overlapping laths organized into distinct zones that are connected through twisting elements at the zonal interfaces. The overall structure has an underlying 57 nm repeating unit. Biomechanical properties inferred from PFR structure lead us to propose that the PFR functions as a biomechanical spring that may store and transmit energy derived from axonemal beating. These findings provide insight into the structural foundations that underlie the distinctive flagellar waveform that is a hallmark of *T. brucei* cell motility.

16246. **Ikeda, K. N. & de Graffenried, C. L., 2012.** Polo-like kinase is necessary for flagellum inheritance in *Trypanosoma brucei*. *Journal of Cell Science*. **Advance online publication 16 March.**

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

Polo-like kinases play an important role in a variety of mitotic events in mammalian cells, ranging from centriole separation and chromosome congression to abscission. To fulfil these roles, PLK homologs move to different cellular locations as the cell cycle progresses, starting at the centrosome, progressing to the spindle poles and then the midbody. In the protist parasite *Trypanosoma brucei*, the single polo-like kinase homolog TbPLK is essential for cytokinesis and is necessary for the correct duplication of a centrin-containing cytoskeletal structure known as the bilobe. We show that TbPLK has a dynamic localization pattern during the cell cycle. The kinase localizes to the basal body, which nucleates the flagellum, and then successively localizes to a series of cytoskeletal structures that regulate the position and attachment of the flagellum to the cell body. The kinase localizes to each of these structures as they are duplicating. TbPLK associates with a specialized set of microtubules, known as the microtubule quartet, which may transport the kinase during its migration. Depletion of TbPLK causes defects in basal body segregation and blocks the duplication of the regulators that position the flagellum, suggesting that its presence on these structures

might be necessary for their proper biogenesis. The ability of PLKs to migrate throughout the cell is preserved in *T. brucei*, but the specific locations to which it targets and functions are geared towards the inheritance of a properly positioned and attached flagellum.

16247. **Izquierdo, L., Mehlert, A. & Ferguson, M. A., 2012.** The lipid-linked oligosaccharide donor specificities of *Trypanosoma brucei* oligosaccharyltransferases. *Glycobiology*, **22** (5): 696-703.

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

16248. **Joice, A. C., Lyda, T. L., Sayce, A. C., Verplaetse, E., Morris, M. T., Michels, P. A., Robinson, D. R. & Morris, J. C., 2012.** Extra-glycosomal localisation of *Trypanosoma brucei* hexokinase 2. *International Journal of Parasitology*, **42** (4): 401-409.

Department of Genetics and Biochemistry, Clemson University, Clemson, SC 39634, USA; Research Unit for Tropical Diseases, de Duve Institute and Laboratory of Biochemistry, Université catholique de Louvain, Brussels, Belgium; and CNRS, University of Bordeaux 2, Bordeaux, France. [jmorri2@clemson.edu].

16249. **Lai, D. H., Bontempi, E. J. & Lukes, J., 2012.** *Trypanosoma brucei* solanesyl-diphosphate synthase localizes to the mitochondrion. *Molecular & Biochemical Parasitology*, **183** (2): 189-192.

Sir William Dunn School of Pathology, University of Oxford, Oxford, OX1 3RE, UK. [ltaclai@hotmail.com].

16250. **Lerch, M., Carnes, J., Acestor, N., Guo, X., Schnaufer, A. & Stuart, K., 2012.** Editosome accessory factors KREPB9 and KREPB10 in *Trypanosoma brucei*. *Eukaryotic Cell*, **11** (7): 832-843.

Seattle Biomedical Research Institute, Seattle, Washington, USA.

16251. **Li, F. J., Shen, Q., Wang, C., Sun, Y., Yuan, A. Y. & He, C. Y., 2012.** A role of autophagy in *Trypanosoma brucei* cell death. *Cellular Microbiology,* **14**(8): 1242-1256.

Department of Biological Sciences Centre for BioImaging Sciences, National University of Singapore, 117543 Singapore.

16252. **Lima, A. H., Souza, P. R., Alencar, N., Lameira, J., Govender, T., Kruger, H. G., Maguire, G. E. & Alves, C. N., 2012.** Molecular modelling of *T. rangeli, T. brucei gambiense*, and *T. evansi* sialidases in complex with the DANA inhibitor. *Chemical Biology & Drug Design*, **80** (1): 114-120.

Laboratorio de Planejamento e Desenvolvimento de Farmacos, Instituto de Ciencias Exatas e Naturais, Universidade Federal do Para, CP 11101, 66075-110, Belem, PA, Brazil; Instituto de Ciencias Biologicas, Universidade Federal do Para, CP 11101, 66075-110, Belem, PA, Brazil; School of Pharmacy and Pharmacology, University of KwaZulu Natal, Durban 4001, South Africa; and School of Chemistry, University of

KwaZulu Natal, Durban 4001, South Africa. [lameira@ufpa.br].

Trypanosomal (trans-) sialidases are enzymes that catalyse the transfer of sialic acid residues between host and parasite glycoconjugates. Herein, we have used homology modelling to construct the 3D structures of sialidases from *Trypanosoma brucei* and *Trypanosoma evansi*. Hybrid quantum mechanical/molecular dynamics simulations were used to determine the interaction energy between the 2-deoxy-2,3-didehydro-N-acetylneuraminic acid inhibitor and the three sialidases studied here. Our results suggest that the two constructed enzymes share the same basic fold motive of the *Trypanosoma rangeli* crystallographic structure. In addition, quantum mechanical/molecular dynamics simulations show that the 2-deoxy-2,3-didehydro-N-acetylneuraminic acid inhibitor forms a stronger complex with *Trypanosoma rangeli* than with *Trypanosoma brucei* and *Trypanosoma evansi* sialidases. Finally, the interaction energy by residues shows that the arginine triad plays a decisive role to complex 2-deoxy-2,3-didehydro-N-acetylneuraminic acid with the enzyme through hydrogen bonding.

16253. **Lima, L., Ortiz, P. A., da Silva, F. M., Alves, J. M., Serrano, M. G., Cortez, A. P., Alfieri, S. C., Buck, G. A. & Teixeira, M. M., 2012.** Repertoire, genealogy and genomic organization of cruzipain and homologous genes in *Trypanosoma cruzi, T. cruzi*-like and other trypanosome species. *PLoS One*, **7** (6): e38385.

Departamento de Parasitologia, ICB, Universidade de Sao Paulo, Sao Paulo, Brazil. [mmgteix@icb.usp.br].

16254. **Macgregor, P. & Matthews, K. R., 2012.** Identification of the regulatory elements controlling the transmission stage-specific gene expression of PAD1 in *Trypanosoma brucei*. *Nucleic Acids Research*. **Published online 7 June.**

Centre for Immunity, Infection and Evolution, Institute for Immunology and Infection Research, School of Biological Sciences, University of Edinburgh, King's Buildings, West Mains Road, Edinburgh, EH9 3JTU, UK. [keith.matthews@ed.ac.uk].

Trypanosomatid parasites provide an extreme model for the posttranscriptional control of eukaryotic gene expression. However, most analysis of their differential gene regulation has focused on comparisons between life cycle stages that exist in the blood of mammalian hosts and tsetse flies, the parasite's vector. These environments differ acutely in their temperature, and nutritional, metabolic and molecular composition. In the bloodstream, however, a more exquisitely regulated developmental step occurs: the production of transmissible stumpy forms from proliferative slender forms. This transition occurs in the relatively homogenous bloodstream environment, with stumpy-specific gene expression being repressed until accumulation of a proposed parasite-derived signal, stumpy induction factor. Here, we have dissected the regulatory signals that repress the expression of the stumpy-specific surface transporter PAD1 in slender forms. Using transgenic parasites capable of stumpy formation we show that PAD1-repression is mediated by its 3'-untranslated region. Dissection of this region in monomorphic slender forms and pleomorphic slender and stumpy forms has revealed that two regulatory regions co-operate to repress PAD1 expression, this being alleviated on exposure to SIF in pleomorphs or cAMP analogues that act as stumpy induction factor mimics in monomorphs. These studies identify elements that regulate trypanosome gene expression during development in their mammalian host.

16255. **May, S. F., Peacock, L., Almeida Costa, C. I., Gibson, W. C., Tetley, L., Robinson, D. R. & Hammarton, T. C., 2012.** The *Trypanosoma brucei* AIR9-like protein is cytoskeleton-associated and is required for nucleus positioning and accurate cleavage furrow placement. *Molecular Microbiology*, **84** (1): 77-92.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK. [Tansy.Hammarton@glasgow.ac.uk].

16256. **McLuskey, K., Rudolf, J., Proto, W. R., Isaacs, N. W., Coombs, G. H., Moss, C. X. & Mottram, J. C., 2012.** Crystal structure of a *Trypanosoma brucei* metacaspase. *Proceedings of the National Academy of Sciences USA*, **109** (19): 7469-7474.

Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity, and Inflammation, College of Medical, Veterinary, and Life Sciences, University of Glasgow, Glasgow G12 8TA, UK. [karen.mcluskey@glasgow.ac.uk].

16257. **Mehlert, A., Wormald, M. R. & Ferguson, M. A., 2012.** Modelling of the N-glycosylated transferrin receptor suggests how transferrin binding can occur within the surface coat of *Trypanosoma brucei*. *PLoS Pathogens*, **8** (4): e1002618.

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

The transferrin receptor of bloodstream form *Trypanosoma brucei* is a heterodimer encoded by expression site associated genes six and seven. This low-abundance glycoprotein with a single glycosylphosphatidylinositol membrane anchor and eight potential N-glycosylation sites is located in the flagellar pocket. The receptor is essential for the parasite, providing its only source of iron by scavenging host transferrin from the bloodstream. Here, we demonstrate that both receptor subunits contain endoglycosidase H-sensitive and endoglycosidase H-resistant N-glycans. Lectin blotting of the purified receptor and structural analysis of the released N-glycans revealed oligomannose and paucimannose structures but, contrary to previous suggestions, no poly-N-acetyllactosamine structures were found. Overlay experiments suggest that the receptor can bind to other trypanosome glycoproteins, which may explain this discrepancy. Nevertheless, these data suggest that a current model, in which poly-N-acetyllactosamine glycans are directly involved in receptor-mediated endocytosis in bloodstream form *Trypanosoma brucei*, should be revised. Sequential endoglycosidase H and peptide-N-glycosidase F treatment, followed by tryptic peptide analysis, allowed the mapping of oligomannose and paucimannose structures to four of the receptor N-glycosylation sites. These results are discussed with respect to the current model for protein N-glycosylation in the parasite. Finally, the glycosylation data allowed the creation of a molecular model for the parasite transferrin receptor. This model, when placed in the context of a model for the dense variant surface glycoprotein coat in which it is embedded, suggests that receptor N-glycosylation may play an important role in providing sufficient space for the approach and binding of transferrin to the receptor, without significantly disrupting the continuity of the protective variant surface glycoprotein coat.

16258. **Mercaldi, G. F., Pereira, H. M., Cordeiro, A. T., Michels, P. A. & Thiemann, O. H., 2012.** Structural role of the active-site metal in the conformation of *Trypanosoma brucei* phosphoglycerate mutase. *FEBS Journal*, **279** (11): 2012-2021.

Instituto de Fisica de Sao Carlos, Grupo de Cristalografia, Universidade de Sao Paulo, Sao Carlos, Sao Paulo, Brazil. [thiemann@ifsc.usp.br].

16259. **Michaeli, S., 2012.** Spliced leader RNA silencing (SLS) - a programmed cell death pathway in *Trypanosoma brucei* that is induced upon ER stress. *Parasites & Vectors*, **5** (1): 107.

The Mina and Everard Goodman Faculty of Life Sciences, and Advanced Materials and Nanotechnology Institute, Bar-Ilan University, Ramat-Gan, 52900, Israel. [shulamit.michaeli@biu.ac.il].

Trypanosoma brucei is the causative agent of African sleeping sickness. The parasite cycles between its insect (procyclic form) and mammalian hosts (bloodstream form). Trypanosomes lack conventional transcription regulation, and their genes are transcribed in polycistronic units that are processed by trans-splicing and polyadenylation. In trans-splicing, which is essential for processing of each mRNA, an exon, the spliced leader (SL) is added to all mRNAs from a small RNA, the SL RNA. Trypanosomes lack the machinery for the unfolded protein response (UPR), which in other eukaryotes is induced under endoplasmic reticulum (ER) stress. Trypanosomes respond to such stress by changing the stability of mRNAs, which are essential for coping with the stress. However, under severe ER stress that is induced by blocking translocation of proteins to the ER, treatment of cells with chemicals that induce misfolding in the ER, or extreme pH, trypanosomes elicit the spliced leader silencing (SLS) pathway. In SLS, the transcription of the SL RNA gene is extinguished, and tSNAP42, a specific SL RNA transcription factor, fails to bind to its cognate promoter. SLS leads to complete shut-off of trans-splicing. This review discusses the UPR in mammals and compares it to the ER stress response in *T. brucei* leading to SLS. Evidence is summarized supporting the notion that SLS is a programmed cell death (PCD) pathway that is utilized by the parasites to substitute for the apoptosis observed in higher eukaryotes under prolonged ER stress. The hypothesis is presented that SLS evolved to expedite the death process, and rapidly removed unfit parasites from the population via SLS, thereby causing minimal damage to the parasite population.

16260. **Michaeli, S., Doniger, T., Gupta, S. K., Wurtzel, O., Romano, M., Visnovezky, D., Sorek, R., Unger, R. & Ullu, E., 2012.** RNA-seq analysis of small RNPs in *Trypanosoma brucei* reveals a rich repertoire of non-coding RNAs. *Nucleic Acids Research*, **40** (3): 1282-1298.

The Mina and Everard Goodman Faculty of Life Sciences, and Advanced Materials and Nanotechnology Institute, Bar-Ilan University, Ramat-Gan 52900, Israel. [michaes@mail.biu.ac.il].

The discovery of a plethora of small non-coding RNAs (ncRNAs) has fundamentally changed our understanding of how genes are regulated. In this study, we employed the power of deep sequencing of RNA (RNA-seq) to examine the repertoire of ncRNAs present in small ribonucleoprotein particles (RNPs) of *Trypanosoma brucei*, an important protozoan parasite. We identified new C/D and H/ACA small nucleolar RNAs (snoRNAs), as well as tens of putative novel non-coding RNAs; several of these are processed from trans-spliced and polyadenylated transcripts. The RNA-seq analysis provided information on the relative abundance of the RNAs, and their 5'- and 3'-termini. The study demonstrated that three highly abundant snoRNAs are involved in rRNA processing and highlight the unique trypanosome-specific repertoire of these RNAs. Novel RNAs were studied using *in situ* hybridization,

association in RNP complexes, and "RNA walk" to detect interaction with their target RNAs. Finally, we showed that the abundance of certain ncRNAs varies between the two stages of the parasite, suggesting that ncRNAs may contribute to gene regulation during the complex parasite's life cycle. This is the first study to provide a whole-genome analysis of the large repertoire of small RNPs in trypanosomes.

16261. **Millerioux, Y., Morand, P., Biran, M., Mazet, M., Moreau, P., Wargnies, M., Ebikeme, C., Deramchia, K., Gales, L., Portais, J. C., Boshart, M., Franconi, J. M. & Bringaud, F., 2012.** ATP synthesis-coupled and -uncoupled acetate production from acetyl-CoA by mitochondrial acetate:succinate CoA-transferase and acetyl-CoA thioesterase in *Trypanosoma*. *Journal of Biological Chemistry*, **287** (21): 17186-17197.

Centre de Résonance Magnétique des Systèmes Biologiques, UMR 5536, Université Bordeaux Segalen, CNRS, 146 Rue Leo Saignat, 33076 Bordeaux, France.

16262. **Ouna, B. A., Stewart, M., Helbig, C. & Clayton, C., 2012.** The *Trypanosoma brucei* CCCH zinc finger proteins ZC3H12 and ZC3H13. *Molecular & Biochemical Parasitology*, **183** (2): 184-188.

Zentrum fur Molekular Biologie Heidelberg, ZMBH-DKFZ Alliance, Im Neuenheimerfeld 282, Heidelberg 69120, Germany. [cclayton@zmbh.uni-heidelberg.de].

16263. **Park, Y. J., Budiarto, T., Wu, M., Pardon, E., Steyaert, J. & Hol, W. G., 2012.** The structure of the C-terminal domain of the largest editosome interaction protein and its role in promoting RNA binding by RNA-editing ligase L2. *Nucleic Acids Research,* **40**(14): 6966-6977.

Biomolecular Structure Center, Department of Biochemistry, School of Medicine, University of Washington, Seattle, WA 98195, USA; Structural Biology Brussels, Vrije Universiteit Brussel and Department of Structural Biology, VIB, Pleinlaan 2, B-1050 Brussels, Belgium. [wghol@u.washington.edu].

Trypanosomatids such as the sleeping sickness parasite *Trypanosoma brucei* contain an approximately 20S RNA-editing complex, also called the editosome, which is required for U-insertion/deletion editing of mitochondrial mRNAs. The editosome contains a core of 12 proteins including the large interaction protein A1, the small interaction protein A6, and the RNA - editing ligase L2. Using biochemical and structural data, we identified distinct domains of *T. brucei* A1 which specifically recognize A6 and L2. We provide evidence that an N-terminal domain of A1 interacts with the C-terminal domain of L2. The C-terminal domain of A1 appears to be required for the interaction with A6 and also plays a key role in RNA binding by the RNA-editing ligase L2 in trans. Three crystal structures of the C-terminal domain of A1 have been elucidated, each in complex with a nanobody as a crystallization chaperone. These structures permitted the identification of putative dsRNA recognition sites. Mutational analysis of conserved residues of the C-terminal domain identified Arg703, Arg731 and Arg734 as key requirements for RNA binding. The data show that the editing RNA ligase activity is modulated by a novel mechanism, i.e. by the trans-acting RNA binding C-terminal domain of A1.

16264. **Park, S. H., Nguyen, T. N. & Gunzl, A., 2012.** Development of an efficient *in vitro*

transcription system for bloodstream form *Trypanosoma brucei* reveals life cycle-independent functionality of class I transcription factor A. *Molecular & Biochemical Parasitology*, **181** (1): 29-36.

Department of Genetics and Developmental Biology, University of Connecticut Health Center, Farmington, CT 06030-6403, USA; and Department of Molecular, Microbial and Structural Biology, University of Connecticut Health Center, 263 Farmington Avenue, Farmington, CT 06030-3305, USA [gunzl@uchc.edu].

Trypanosomatid parasites possess extremely divergent transcription factors whose identification typically relied on biochemical, structural and functional analyses because they could not be identified by standard sequence analysis. For example, subunits of the *Trypanosoma brucei* mediator and class I transcription factor A (CITFA) have no sequence resemblance to putative counterparts in higher eukaryotes. Therefore, homologous *in vitro* transcription systems have been crucial in evaluating the transcriptional roles of *T. brucei* proteins but so far such systems have been restricted to the insect-stage, procyclic form (PF) of the parasite. Here, we report the development of a homologous system for the mammalian-infective, bloodstream form (BF) of *T. brucei* which supports accurately initiated transcription from three different RNA polymerase (pol) I promoters as well as from the RNA pol II-recruiting spliced leader RNA gene promoter. The system is based on a small scale extract preparation procedure which accommodates the low cell densities obtainable in BF culture. BF and PF systems behave surprisingly similar and we show that the CITFA complex purified from procyclic extract is fully functional in the BF system indicating that the transcriptional machinery in general is equivalent in both life cycle stages. A notable difference, however, was observed with the RNA pol I-recruiting GPEET procyclin promoter whose reduced promoter strength and increased sensitivity to manganese ions in the BF system suggest the presence of a specific transcriptional activator in the PF system.

16265. **Poon, S. K., Peacock, L., Gibson, W., Gull, K. & Kelly, S., 2012.** A modular and optimized single marker system for generating *Trypanosoma brucei* cell lines expressing T7 RNA polymerase and the tetracycline repressor. *Open Biology*, **2** (2): 110037.

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE , UK; Oxford Centre for Integrative Systems Biology, Department of Biochemistry, University of Oxford, South Parks Road, Oxford OX1 3QU, UK; School of Biological Sciences, University of Bristol, Bristol BS8 1UG, UK; Centre for Mathematical Biology, Mathematical Institute, University of Oxford, 24-29 St Giles', Oxford OX1 3LB, UK; and Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, UK. [steven.kelly@plants.ox.ac.uk].

Here, we present a simple modular extendable vector system for introducing the T7 RNA polymerase and tetracycline repressor genes into *Trypanosoma brucei*. This novel system exploits developments in our understanding of gene expression and genome organization to produce a streamlined plasmid optimized for high levels of expression of the introduced transgenes. We demonstrate the utility of this novel system in bloodstream and procyclic forms of *Trypanosoma brucei*, including the genome strain TREU927/4. We validate these cell lines using a variety of inducible experiments that recapture previously published lethal and non-lethal phenotypes. We further demonstrate the utility of the single marker (SmOx) TREU927/4 cell line for *in vivo* experiments in the tsetse fly and provide a set of plasmids that enable both whole-fly and salivary gland-specific inducible expression of transgenes.

16266. **Portman, N. & Gull, K., 2012.** Proteomics and the *Trypanosoma brucei* cytoskeleton: advances and opportunities. *Parasitology*: 1-10.

The Sir William Dunn School of Pathology and Oxford Centre for Integrative Systems Biology, University of Oxford, South Parks Road, Oxford, OX1 3RE, UK. [keith.gull@path.ox.ac.uk].

Trypanosoma brucei is the aetiological agent of devastating parasitic diseases in humans and livestock in sub-Saharan Africa. The pathogenicity and growth of the parasite are intimately linked to its shape and form. This is in turn derived from a highly ordered microtubule cytoskeleton that forms a tightly arrayed cage directly beneath the pellicular membrane and numerous other cytoskeletal structures such as the flagellum. The parasite undergoes extreme changes in cellular morphology during its life cycle and cell cycles which require a high level of integration and coordination of cytoskeletal processes. In this review we discuss the role that proteomics techniques have had in advancing our understanding of the molecular composition of the cytoskeleton and its functions. We then consider future opportunities for the application of these techniques in terms of addressing some of the unanswered questions of trypanosome cytoskeletal cell biology with particular focus on the differences in the composition and organisation of the cytoskeleton through the trypanosome life cycle.

16267. **Price, H. P., Hodgkinson, M. R., Curwen, R. S., MacLean, L. M., Brannigan, J. A., Carrington, M., Smith, B. A., Ashford, D. A., Stark, M. & Smith, D. F., 2012.** The orthologue of Sjogren's syndrome nuclear autoantigen 1 (SSNA1) in *Trypanosoma brucei* is an immunogenic self-assembling molecule. *PLoS One*, **7** (2): e31842.

Centre for Immunology and Infection, Department of Biology, University of York, Heslington, York, UK. [helen.price@york.ac.uk].

16268. **Price, H. P., Hodgkinson, M. R., Wright, M. H., Tate, E. W., Smith, B. A., Carrington, M., Stark, M. & Smith, D. F., 2012.** A role for the vesicle-associated tubulin binding protein ARL6 (BBS3) in flagellum extension in *Trypanosoma brucei*. *Biochimica et Biophysica Acta*, **1823** (7): 1178-1191.

Centre for Immunology and Infection, Department of Biology, University of York, Heslington, York YO10 5YW, UK. [helen.price@york.ac.uk].

16269. **Ramasamy, R. & Field, M. C., 2012.** Terminal galactosylation of glycoconjugates in *Plasmodium falciparum* asexual blood stages and *Trypanosoma brucei* bloodstream trypomastigotes. *Experimental Parasitology*, **130** (4): 314-320.

Department of Pathology, University of Cambridge, Cambridge, UK. [ranjan.ramasamy@ubd.edu.bn].

There is definitive biochemical evidence for the presence of terminal alpha-galactosyl residues (alpha-gal) in the N-linked oligosaccharides and glycophosphatidylinositol anchors (GPI anchors) of the variant surface glycoprotein of *Trypanosoma brucei* bloodstream trypomastigotes. Indirect evidence also exists for alpha-gal in *Plasmodium falciparum* asexual blood stage glycoproteins and glycolipids. The occurrence of alpha-gal in glycoproteins and

glycolipids of *T. brucei* bloodstream trypomastigotes and *P. falciparum* late asexual blood stages was investigated by the binding of alpha-gal-specific *Bandeirea simplicifolia* B4 lectin 1 (BSB4), incorporation of [^{3}H]galactose from UDP-[^{3}H]galactose into glycoproteins and glycolipids in microsomes *in vitro*, and bioinformatic searches for galactosyl-transferase coding sequences. The findings confirm the presence of alpha-gal in a spectrum of *T. brucei* bloodstream trypomastigote glycoproteins and glycolipids and indicate its relative absence from *P. falciparum* asexual blood stage glycoconjugates.

16270. **Ranjbarian, F., Vodnala, M., Vodnala, S. M., Rofougaran, R., Thelander, L. & Hofer, A., 2012.** *Trypanosoma brucei* thymidine kinase is tandem protein consisting of two homologous parts, which together enable efficient substrate binding. *Journal of Biological Chemistry*, **287** (21): 17628-17636.

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

16271. **Rettig, J., Wang, Y., Schneider, A. & Ochsenreiter, T., 2012.** Dual targeting of isoleucyl-tRNA synthetase in *Trypanosoma brucei* is mediated through alternative trans-splicing. *Nucleic Acids Research*, **40** (3): 1299-1306.

Department of Chemistry and Biochemistry, University of Bern, Freiestrasse 3, 3012 Bern, Switzerland. [andre.schneider@ibc.unibe.ch].

16272. **Salmon, D., Vanwalleghem, G., Morias, Y., Denoeud, J., Krumbholz, C., Lhomme, F., Bachmaier, S., Kador, M., Gossmann, J., Dias, F. B., De Muylder, G., Uzureau, P., Magez, S., Moser, M., De Baetselier, P., Van Den Abbeele, J., Beschin, A., Boshart, M. & Pays, E., 2012.** Adenylate cyclases of *Trypanosoma brucei* inhibit the innate immune response of the host. *Science,* **337**(6093): 463-466.

Laboratory of Molecular Parasitology, IBMM, Université Libre de Bruxelles, 12, rue des Prof. Jeener et Brachet, B6041 Gosselies, Belgium; Institute of Medical Biochemistry, Centro de Ciências e da Saude, Federal University of Rio de Janeiro, Avenida General Trompowsky, Rio de Janeiro 21941-590, Brazil; Myeloid Cell Immunology Laboratory, Vlaams Instituut voor Biotechnologie, Brussels, Belgium; Cellular and Molecular Immunology Unit, Vrije Universiteit Brussel, Brussels, Belgium; Laboratory of Immunobiology, IBMM, Université Libre de Bruxelles, Gosselies, Belgium; Biocenter, Section Genetics, Ludwig-Maximilians-Universität München, Martinsried, Germany; Center for Microscopy and Molecular Imaging, Gosselies, Belgium; Department of Structural Biology, VIB, Brussels, Belgium; Department of Biomedical Sciences, Unit of Veterinary Protozoology, Institute of Tropical Medicine Antwerp, Antwerp, Belgium; and Walloon Excellence in Life Sciences and Biotechnology (WELBIO), Wavre, Belgium. [salmon@bioqmed.ufrj.br].

The parasite *Trypanosoma brucei* possesses a large family of transmembrane receptor-like adenylate cyclases. Activation of these enzymes requires the dimerization of the catalytic domain and typically occurs under stress. Using a dominant-negative strategy, we found that reducing adenylate cyclase activity by ~50 percent allowed trypanosome growth but reduced the parasite's ability to control the early innate immune defense of the host. Specifically, activation of trypanosome adenylate cyclase resulting from parasite phagocytosis by liver myeloid cells inhibited the synthesis of the trypanosome-controlling cytokine tumour necrosis factor-alpha through activation of protein kinase A in these cells. Thus, adenylate cyclase

activity of lysed trypanosomes favours early host colonization by live parasites. The role of adenylate cyclases at the host-parasite interface could explain the expansion and polymorphism of this gene family.

16273. **Seidman, D., Johnson, D., Gerbasi, V., Golden, D., Orlando, R. & Hajduk, S., 2012.** Mitochondrial membrane complex that contains proteins necessary for tRNA import in *Trypanosoma brucei*. *Journal of Biological Chemistry*, **287** (12): 8892-8903.

Department of Biochemistry and Molecular Biology, University of Georgia, Athens, Georgia 30602-7229, USA.

The mitochondrial genome of *Trypanosoma brucei* does not contain genes encoding tRNAs; instead this protozoan parasite must import nuclear-encoded tRNAs from the cytosol for mitochondrial translation. Previously, it has been shown that mitochondrial tRNA import requires ATP hydrolysis and a proteinaceous mitochondrial membrane component. However, little is known about the mitochondrial membrane proteins involved in tRNA binding and translocation into the mitochondrion. Here we report the purification of a mitochondrial membrane complex using tRNA affinity purification and have identified several protein components of the putative tRNA translocon by mass spectrometry. Using an *in vivo* tRNA import assay in combination with RNA interference, we have verified that two of these proteins, Tb11.01.4590 and Tb09.v1.0420, are involved in mitochondrial tRNA import. Using protein C epitope -tobacco etch virus-protein A epitope (PTP)-tagged Tb11.01.4590, additional associated proteins were identified including Tim17 and other mitochondrial proteins necessary for mitochondrial protein import. Results presented here identify and validate two novel protein components of the putative tRNA translocon and provide additional evidence that mitochondrial tRNA and protein import have shared components in trypanosomes.

16274. **Serricchio, M. & Butikofer, P., 2012.** An essential bacterial-type cardiolipin synthase mediates cardiolipin formation in a eukaryote. *Proceedings of the National Academy of Sciences USA*, **109** (16): E954-961.

Institute of Biochemistry and Molecular Medicine, University of Bern, 3012 Bern, Switzerland. [peter.buetikofer@ibmm.unibe.ch].

Cardiolipin is important for bacterial and mitochondrial stability and function. The final step in cardiolipin biosynthesis is catalysed by cardiolipin synthase and differs mechanistically between prokaryotes and eukaryotes. To study the importance of cardiolipin synthesis for mitochondrial integrity, membrane protein complex formation, and cell proliferation in the human and animal pathogenic protozoan parasite, *Trypanosoma brucei*, we generated conditional cardiolipin synthase-knockout parasites. We found that cardiolipin formation in *T. brucei* procyclic forms is catalysed by a bacterial-type cardiolipin synthase, providing experimental evidence for a prokaryotic-type cardiolipin synthase in a eukaryotic organism. Ablation of enzyme expression resulted in inhibition of *de novo* cardiolipin synthesis, reduction in cellular cardiolipin levels, alterations in mitochondrial morphology and function, and parasite death in culture. By using immunofluorescence microscopy and blue-native gel electrophoresis, cardiolipin synthase was shown to colocalize with inner mitochondrial membrane proteins and to be part of a large protein complex. During depletion of cardiolipin synthase, the levels of cytochrome oxidase subunit IV and cytochrome c1, reflecting mitochondrial respiratory complexes IV and III, respectively, decreased progressively.

16275. **Simmons, J. M., Koslowsky, D. J. & Hausinger, R. P., 2012.** Characterization of a *Trypanosoma brucei* Alkb homolog capable of repairing alkylated DNA. *Experimental Parasitology*, **131** (1): 92-100.

Department of Biochemistry & Molecular Biology, Michigan State University, East Lansing, MI, USA; and Department of Microbiology & Molecular Genetics, Michigan State University, East Lansing, MI, USA. [hausinge@msu.edu].

16276. **Singha, U. K., Hamilton, V., Duncan, M. R., Weems, E., Tripathi, M. K. & Chaudhuri, M., 2012.** Protein translocase of mitochondrial inner membrane in *Trypanosoma brucei*. *Journal of Biological Chemistry*, **287** (18): 14480-14493.

Department of Microbiology and Immunology, Meharry Medical College, Nashville, Tennessee 37208, USA.

16277. **Solnoki, K. W., Sing, A. H., Sofa, C. J., Miller, R., Ogorzalek, P. A., Penek, H. V. & Palenchar, J. B., 2012.** TbENF is an essential TbTFIIB-interacting trypanosomatid-specific factor. *Molecular & Biochemical Parasitology*, **181** (2): 94-101.

Department of Chemistry, Villanova University, Villanova, PA 19085, USA. [jennifer.palenchar@villanova.edu].

Trypanosoma brucei, the causative agent of African sleeping sickness, is replete with unique biochemistry, including unusual features of gene transcription. The parasite also contains over 4 500 non-annotated genes, representing novel biochemistry yet to be explored. Using tandem affinity purification (TAP)-tagged TbTFIIB, we identified and subsequently confirmed one of the non-annotated *T. brucei* proteins, Tb11.02.4300, as a TbTFIIB-interacting protein. The 49 kDa protein is nuclear and essential for parasite variability as determined by RNA interference studies; hence, the nomenclature *T. brucei* essential nuclear factor (TbENF). TbENF is shown to interact with DNA in a sequence-independent fashion under the conditions examined. Furthermore, TbENF bears motifs associated with many eukaryotic transcription factors, such as a glutamine-rich region and a leucine zipper, yet TbENF is specific to trypanosomatids making it a potentially attractive therapeutic target. Taken together, our results suggest a role for TbENF in trypanosome gene transcription.

16278. **Sunter, J., Wickstead, B., Gull, K. & Carrington, M., 2012.** A new generation of T7 RNA polymerase-independent inducible expression plasmids for *Trypanosoma brucei*. *PLoS One*, **7** (4): e35167.

Department of Biochemistry, University of Cambridge, Cambridge, UK; Centre for Genetics and Genomics, University of Nottingham, Nottingham, UK; and Sir William Dunn School of Pathology, University of Oxford, Oxford, UK. [mc115@cam.ac.uk9].

Expression of transgenes is central to forward and reverse genetic analysis in *Trypanosoma brucei*. The inducible expression of transgenes in trypanosomes is based on the tetracycline repressor binding to a tetracycline operator to prevent transcription in the absence of tetracycline. The same inducible system is used to produce double-stranded RNA for RNAi knockdown of target genes. This study describes a new plasmid pSPR2.1 that drives consistent high-level expression of tetracycline repressor in procyclic form trypanosomes. A

complementary expression plasmid, p3227, was constructed. The major difference between this and current plasmids is the separation of the inducible transgene and selectable marker promoters by the plasmid backbone. The plasmid p3227 was able to support inducible expression in cell lines containing pSPR2.1 as well as in the established Lister 427 29-13 cell line. p3666, a derivative of p3227, was made for inducible expression of stem loop RNAi constructs and was effective for knockdown of DRBD3, which had proved problematic using existing RNAi plasmids with head-to-head promoters. The plasmid system was also able to support inducible transgene expression and DRBD3 RNAi knockdown in bloodstream form cells expressing tetracycline repressor from an integrated copy of the plasmid pHD1313.

16279. **Surve, S., Heestand, M., Panicucci, B., Schnaufer, A. & Parsons, M., 2012.** Enigmatic presence of mitochondrial complex I in *Trypanosoma brucei* bloodstream forms. *Eukaryotic Cell*, **11** (2): 183-193.

Seattle Biomedical Research Institute, Seattle, Washington, USA. [achim.schnaufer@ed.ac.uk].

16280. **Tiengwe, C., Marcello, L., Farr, H., Gadelha, C., Burchmore, R., Barry, J. D., Bell, S. D. & McCulloch, R., 2012.** Identification of ORC1/CDC6-interacting factors in *Trypanosoma brucei* reveals critical features of origin recognition complex architecture. *PLoS One*, **7** (3): e32674.

The Wellcome Trust Centre for Molecular Parasitology, Institute of Infection, Immunity and Inflammation, University of Glasgow, Glasgow, UK. [richard.mcculloch@glasgow.ac.uk].

DNA replication initiates by formation of a pre-replication complex on sequences termed origins. In eukaryotes, the pre-replication complex is composed of the origin recognition complex (ORC), Cdc6 and the MCM replicative helicase in conjunction with Cdt1. Eukaryotic ORC is considered to be composed of six subunits, named Orc1-6, and monomeric Cdc6 is closely related in sequence to Orc1. However, ORC has been little explored in protists, and only a single ORC protein, related to both Orc1 and Cdc6, has been shown to act in DNA replication in *Trypanosoma brucei*. Here we identify three highly diverged putative *T. brucei* ORC components that interact with ORC1/CDC6 and contribute to cell division. Two of these factors are so diverged that we cannot determine if they are eukaryotic ORC subunit orthologues, or are parasite-specific replication factors. The other we show to be a highly diverged Orc4 orthologue, demonstrating that this is one of the most widely conserved ORC subunits in protists and revealing it to be a key element of eukaryotic ORC architecture. Additionally, we have examined interactions amongst the *T. brucei* MCM subunits and show that these have the conventional eukaryotic heterohexameric structure, suggesting that divergence in the *T. brucei* replication machinery is limited to the earliest steps in origin licensing.

16281. **Tsaousis, A. D., Ollagnier de Choudens, S., Gentekaki, E., Long, S., Gaston, D., Stechmann, A., Vinella, D., Py, B., Fontecave, M., Barras, F., Lukes, J. & Roger, A. J., 2012.** Evolution of Fe/S cluster biogenesis in the anaerobic parasite *Blastocystis*. *Proceedings of the National Academy of Sciences USA*, **109** (26): 10426-10431.

Centre for Comparative Genomics and Evolutionary Bioinformatics, Department of Biochemistry and Molecular Biology, Dalhousie University, Halifax, NS, Canada B3H 4R2. [tsaousis.anastasios@gmail.com].

16282. **Urbaniak, M. D., Guther, M. L. & Ferguson, M. A., 2012.** Comparative SILAC proteomic analysis of *Trypanosoma brucei* bloodstream and procyclic life cycle stages. *PLoS One*, **7** (5): e36619.

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

The protozoan parasite *Trypanosoma brucei* has a complex digenetic life cycle between a mammalian host and an insect vector, and adaption of its proteome between lifecycle stages is essential to its survival and virulence. We have optimized a procedure for growing *Trypanosoma brucei* procyclic form cells in conditions suitable for stable isotope labelling by amino acids in culture (SILAC) and report a comparative proteomic analysis of cultured procyclic form and bloodstream form *T. brucei* cells. In total we were able to identify 3 959 proteins and quantify SILAC ratios for 3 553 proteins with a false discovery rate of 0.01. A large number of proteins (10.6 percent) are differentially regulated by more the five-fold between life cycle stages, including those involved in the parasite surface coat, and in mitochondrial and glycosomal energy metabolism. Our proteomic data are broadly in agreement with transcriptomic studies, but with significantly larger fold changes observed at the protein level than at the mRNA level.

16283. **Vacchina, P., Tripodi, K. E., Escalante, A. M. & Uttaro, A. D., 2012.** Characterization of bifunctional sphingolipid delta4-desaturases/C4-hydroxylases of trypanosomatids by liquid chromatography-electrospray tandem mass spectrometry. *Molecular & Biochemical Parasitology*, **184** (1): 29-38.

Instituto de Biologia Molecular y Celular de Rosario (IBR), CONICET, Facultad de Ciencias Bioquimicas y Farmaceuticas, Universidad Nacional de Rosario, Santa Fe, Argentina.

16284. **Vigueira, P. A. & Paul, K. S., 2012.** *Trypanosoma brucei*: inhibition of acetyl-CoA carboxylase by haloxyfop. *Experimental Parasitology*, **130** (2): 159-165.

Department of Biological Sciences, Clemson University, Clemson, SC 29634, USA. [pvigueir@dom.wustl.edu].

16285. **Wang, J., Englund, P. T. & Jensen, R. E., 2012.** TbPIF8, a *Trypanosoma brucei* protein related to the yeast Pif1 helicase, is essential for cell viability and mitochondrial genome maintenance. *Molecular Microbiology*, **83** (3): 471-485.

Departments of Biological Chemistry Cell Biology, Johns Hopkins Medical School, Baltimore, MD 21205, USA. [robjensen@jhmi.edu].

The trypanosome mitochondrial genome, kinetoplast DNA (kDNA), is a massive network of interlocked DNA rings, including several thousand minicircles and dozens of maxicircles. The unusual complexity of kDNA would indicate that numerous proteins must be involved in its condensation, replication, segregation and gene expression. During our investigation of trypanosome mitochondrial PIF1-like helicases, we found that TbPIF8 is the smallest and most divergent. It lacks some conserved helicase domains, thus implying that unlike other mitochondrial PIF1-like helicases, this protein may have no enzymatic activity. TbPIF8 is positioned on the distal face of kDNA disk and its localization patterns vary with different

kDNA replication stages. Stem-loop RNAi of TbPIF8 arrests cell growth and causes defects in kDNA segregation. RNAi of TbPIF8 causes only limited kDNA shrinkage but the networks become disorganized. Electron microcopy of thin sections of TbPIF8-depleted cells shows heterogeneous electron densities in the kinetoplast disk. Although we do not yet know its exact function, we conclude that TbPIF8 is essential for cell viability and is important for maintenance of kDNA.

16286 **Wang, M., Gheiratmand, L. & He, C. Y., 2012.** An interplay between Centrin2 and Centrin4 on the bi-lobed structure in *Trypanosoma brucei*. *Molecular Microbiology*, **83** (6): 1153-1161.

Department of Biological Sciences, National University of Singapore, Singapore. [dbshyc@nus.edu.sg].

16287. **Weisse, S., Heddergott, N., Heydt, M., Pflasterer, D., Maier, T., Haraszti, T., Grunze, M., Engstler, M. & Rosenhahn, A., 2012.** A quantitative 3D motility analysis of *Trypanosoma brucei* by use of digital in-line holographic microscopy. *PLoS One*, **7** (5): e37296.

Applied Physical Chemistry, University of Heidelberg, Heidelberg, Germany. [axel.rosenhahn@urz.uni-heidelberg.de].

We present a quantitative 3D analysis of the motility of the blood parasite *Trypanosoma brucei*. Digital in-line holographic microscopy has been used to track single cells with high temporal and spatial accuracy to obtain quantitative data on their behaviour. Comparing bloodstream form and insect form trypanosomes as well as mutant and wildtype cells under varying external conditions we were able to derive a general two-state-run-and-tumble-model for trypanosome motility. Differences in the motility of distinct strains indicate that adaption of the trypanosomes to their natural environments involves a change in their mode of swimming.

16288. **Wen, Y. Z., Zheng, L. L., Qu, L. H., Ayala, F. J. & Lun, Z. R., 2012.** Pseudogenes are not pseudo any more. *RNA Biology*, **9** (1): 27-32.

School of Life Sciences and Key Laboratory of Tropical Diseases and Control of the Ministry of Education, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, China.

Recent significant progress toward understanding the function of pseudogenes in protozoa (*Trypanosoma brucei*), metazoa (mouse) and plants, make it pertinent to provide a brief overview on what has been learned about this fascinating subject. We discuss the regulatory mechanisms of pseudogenes at the post-transcriptional level and advance new ideas toward understanding the evolution of these, sometimes called "garbage genes" or "junk DNA" seeking to stimulate the interest of scientists and additional research on the subject. We hope this point of view can be helpful to scientists working or seeking to work on these and related issues.

16289. **Wheeler, R. J., Gull, K. & Gluenz, E., 2012.** Detailed interrogation of trypanosome cell biology via differential organelle staining and automated image analysis. *BMC Biology*, **10**: 1.

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

Many trypanosomatid protozoa are important human or animal pathogens. The well-defined morphology and precisely choreographed division of trypanosomatid cells makes morphological analysis a powerful tool for analysing the effect of mutations, chemical insults and changes between lifecycle stages. High-throughput image analysis of micrographs has the potential to accelerate collection of quantitative morphological data. Trypanosomatid cells have two large DNA-containing organelles, the kinetoplast (mitochondrial DNA) and nucleus, which provide useful markers for morphometric analysis; however they need to be accurately identified and often lie in close proximity. This presents a technical challenge. Accurate identification and quantitation of the DNA content of these organelles is a central requirement of any automated analysis method. We have developed a technique based on double staining of the DNA with a minor groove binding (4", 6-diamidino-2-phenylindole (DAPI)) and a base pair intercalating (propidium iodide (PI) or SYBR green) fluorescent stain and colour deconvolution. This allows the identification of kinetoplast and nuclear DNA in the micrograph based on whether the organelle has DNA with a more A-T or G-C rich composition. Following unambiguous identification of the kinetoplasts and nuclei the resulting images are amenable to quantitative automated analysis of kinetoplast and nucleus number and DNA content. On this foundation we have developed a demonstrative analysis tool capable of measuring kinetoplast and nucleus DNA content, size and position and cell body shape, length and width automatically. Our approach to DNA staining and automated quantitative analysis of trypanosomatid morphology accelerated analysis of trypanosomatid protozoa. We have validated this approach using *Leishmania mexicana, Crithidia fasciculata* and wild-type and mutant *Trypanosoma brucei*. Automated analysis of *T. brucei* morphology was of comparable quality to manual analysis while being faster and less susceptible to experimental bias. The complete data set from each cell and all analysis parameters used can be recorded ensuring repeatability and allowing complete data archiving and reanalysis.

16290. **Willert, E. & Phillips, M. A., 2012.** Regulation and function of polyamines in African trypanosomes. *Trends in Parasitology*, **28** (2): 66-72.

Department of Pharmacology, University of Texas Southwestern Medical Center at Dallas, 6001 Forest Park Road, Dallas, TX 75390-9041, USA. [margaret.phillips@UTSouthwestern.edu].

The polyamine biosynthetic pathway is an important drug target for the treatment of human African trypanosomiasis (HAT), raising interest in understanding polyamine function and their mechanism of regulation. Polyamine levels are tightly controlled in mammalian cells, but similar regulatory mechanisms appear absent in trypanosomes. Instead trypanosomatid S-adenosylmethionine decarboxylase (AdoMetDC), which catalyzes a key step in the biosynthesis of the polyamine spermidine, is activated by dimerization with an inducible protein termed prozyme. Prozyme is an inactive paralog of the active AdoMetDC enzyme that evolved by gene duplication and is found only in the trypanosomatids. In *Trypanosoma brucei*, AdoMetDC activity appears to be controlled by regulation of prozyme protein levels, potentially at the translational level.

16291. **Wurst, M., Seliger, B., Jha, B. A., Klein, C., Queiroz, R. & Clayton, C., 2012.** Expression of the RNA recognition motif protein RBP10 promotes a bloodstream-form transcript pattern in *Trypanosoma brucei*. *Molecular Microbiology*, **83** (5): 1048-1063.

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

When *Trypanosoma brucei* differentiates from the bloodstream form to the procyclic form, there are decreases in the levels of many mRNAs encoding proteins required for the glycolytic pathway, and the mRNA encoding the RNA recognition motif protein RBP10 decreases in parallel. We show that RBP10 is a cytoplasmic protein that is specific to bloodstream-form trypanosomes, where it is essential. Depletion of RBP10 caused decreases in many bloodstream-form-specific mRNAs, with increases in mRNAs associated with the early stages of differentiation. The changes were similar to, but more extensive than, those caused by glucose deprivation. Conversely, forced RBP10 expression in procyclics induced a switch towards bloodstream-form mRNA expression patterns, with concomitant growth inhibition. Forced expression of RBP10 prevented differentiation of bloodstream forms in response to cis-aconitate, but did not prevent expression of key differentiation markers in response to glucose deprivation. RBP10 was not associated with heavy polysomes, showed no detectable *in vivo* binding to RNA, and was not stably associated with other proteins. Tethering of RBP10 to a reporter mRNA inhibited translation, and halved the abundance of the bound mRNA. We suggest that RBP10 may prevent the expression of regulatory proteins that are specific to the procyclic form.

16292. **Yu, Z., Liu, Y. & Li, Z., 2012.** Structure-function relationship of the polo-like kinase in *Trypanosoma brucei*. *Journal of Cell Science*, **125** (Pt 6): 1519-1530.

Department of Microbiology and Molecular Genetics, University of Texas Medical School at Houston, 6431 Fannin Street, Houston, TX 77030, USA. [ziyin.li@uth.tmc.edu].

Polo-like kinases (Plks) play multiple roles in mitosis and cytokinesis in eukaryotes and are characterized by the C-terminal polo-box domain (PBD), which is implicated in binding to Plk substrates, targeting Plk and regulating Plk activity. The Plk homolog in *Trypanosoma brucei* (TbPLK) possesses a similar architecture, but it lacks the crucial residues involved in substrate binding and regulates cytokinesis but not mitosis. Little is known about the regulation of TbPLK and the role of the PBD in TbPLK localization and function. Here, we addressed the requirement of the kinase activity and the PBD for TbPLK localization and function through coupling RNAi of endogenous TbPLK with ectopic expression of TbPLK mutants. We demonstrate that the kinase activity and phosphorylation of two threonine residues, Thr198 and Thr202, in the activation loop (T-loop) of the kinase domain are essential for TbPLK function but not for TbPLK localization. Deletion of the PBD abolishes TbPLK localization, but the PBD itself is not correctly targeted, indicating that TbPLK localization requires both the PBD and the kinase domain. Surprisingly, the kinase domain of TbPLK, but not the PBD, binds to its substrates TbCentrin2 and p110, suggesting that TbPLK might interact with its substrate through different mechanisms. Finally, the PBD interacts with the kinase domain of TbPLK and inhibits its activity, and this inhibition is relieved when Thr198 is phosphorylated. Together, these results suggest an essential role of T-loop phosphorylation in TbPLK activation and crucial roles of the PBD in regulating TbPLK activity and localization.

16293. **Zimmer, S. L., McEvoy, S. M., Menon, S. & Read, L. K., 2012.** Additive and transcript-specific effects of KPAP1 and TbRND activities on 3' non-encoded tail characteristics and mRNA stability in *Trypanosoma brucei*. *PLoS One*, **7** (5): e37639.

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

ISBN 978-92-5-107434-3 ISSN 1812-2442

9 789251 074343

I3145E/1/11.12